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ON THE ACROSOME OF THE ANIMAL SPERM

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TWO FIGURES

In a previous paper in this journal (Bowen, '22 a), I undertook a synthesis of the much-confused phenomena which lead up to the formation and deposition of the acrosome in the animal sperm. In that discussion little effort was made to go beyond the actual deposition of the acrosome, since several important points connected with its further fate were still in doubt. Recently, however, much new light has been shed on these doubtful points, and the problem of the acrosome seems now in a fair way of solution. The one point upon which we are still quite ignorant is the fate of the acrosome (morphologically speaking) in the egg after the sperm has once penetrated its cortex. It may, therefore, prove of interest to attempt a synthesis of the final stages in the modeling of the acrosome, thus completing the discussion so far as the sperm itself is concerned. As before, the discussion will be limited primarily to the typical flagellate sperms.

In my previous paper attention was called to two types of construction which seem to characterize the acrosome during its period of formation—the vesicular type and the granular type. In the case of the former, a darkly staining granule is usually developed within the acrosomal vesicle proper—a differentiation which, in the stages following the deposition of the acrosome, is usually obscured. Since the meaning of these two components is unknown, the acrosome will be considered in the following account merely as one simple mass.

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The acrosomal material is deposited by the acroblast (Golgi complex) at some point on the nuclear membrane, usually before the nucleus has changed (in case the sperm head is to be elongate) from its original spherical shape. If this material is not originally in an anterior position, it usually moves at once toward the side of the nucleus which will eventually be the advancing end of the mature sperm. This arrangement is perhaps subject to variations, but it seems nevertheless to be a customary step in sperms whose developmental stages have been followed. We may then consider such an arrangement of parts as the primitive one, from which subsequent departures in structural detail can be derived. Such a simple type of sperm is shown in figure 1*A*. The nucleus retains its spherical form in the mature condition, and the acrosomal material is molded into a small apical piece which may be more or less pointed or in some cases spread out in a thin, flattened layer. Occasionally it may be somewhat drawn out to form a long, thread-like point. In other words, the shape of the sperm head and the relation of the acrosome to it are the important points, while the exact modeling of the acrosome is subject to endless variety and is presumably of no importance—a point of some interest in the light of the perforating function usually attributed to this body. Our knowledge of acrosomes of this primitive type is based largely on the study of mature sperms (see particularly Retzius, '04, '05), and an examination of the developmental stages might conceivably require some modification in the presumed extent and mode of attachment of the acrosome. This type of acrosome is common among annelids, molluscs, and echinoderms.

Starting from this simple, presumably primitive condition, in a large group of sperms the acrosome retains its insertion at the anterior end of the nucleus—remains in fact completely, or almost completely, anterior to it—while the nucleus itself is drawn out in the direction of the major axis of the sperm. The nucleus may thus pass from the original spherical shape of figure 1A through various kinds of cones or ellipsoids (Retzius, '04) to a cylindrical form, which in the simplest

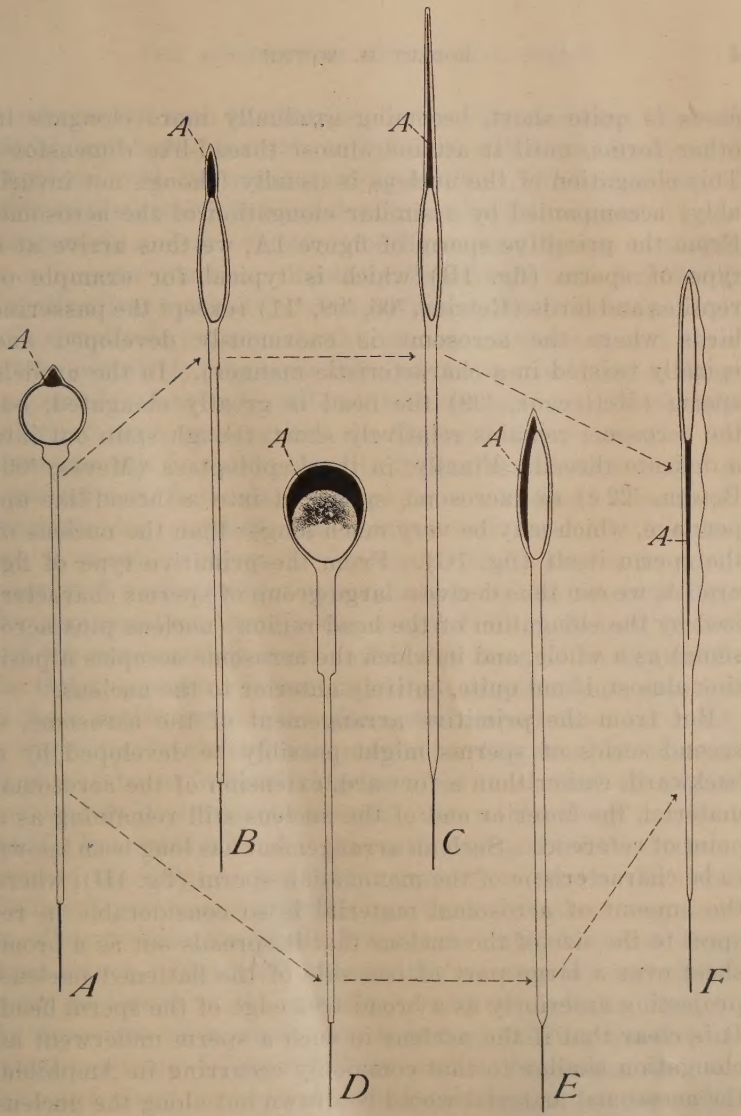

Fig. 1 Diagram illustrating various arrangements of the acrosome with respect to the nucleus of the sperm. The acrosome, *A*, is represented in black; the nucleus and the general contour of the sperm are indicated in outline. The figures are very much schematized, and the connecting lines are intended merely to indicate morphological relations, and to them no evolutionary significance is to be attached. *A*, supposedly primitive disposition of the acrosome, typical of many Annelida, Echinodermata, and Mollusca; *B*, Reptilia, Aves, and Amphibia; *C*, Lepidoptera; *D*, Mammalia; *E*, many Hemiptera and Coleoptera; *F*, Aptera.

cases is quite short, becoming gradually more elongate in other forms, until it attains almost thread-like dimensions. This elongation of the nucleus is usually (though not invariably) accompanied by a similar elongation of the acrosome. From the primitive sperm of figure 1A, we thus arrive at a type of sperm (fig. 1B) which is typical for example of reptiles and birds (Retzius, '06, '09, '11) (except the passerine birds where the acrosome is enormously developed and spirally twisted in a characteristic manner). In the urodele sperm (McGregor, '99) the head is greatly elongated, but the acrosome remains relatively short, though spun out into a delicate thread. Finally, in the Lepidoptera (Meves, '03; Bowen, '22 c) the acrosome spins out into a thread-like appendage, which may be very much longer than the nucleus of the sperm itself (fig. 1C). From the primitive type of figure 1A, we can thus derive a large group of sperms characterized by the elongation of the head region (nucleus plus acrosome) as a whole, and in which the acrosome occupies a position almost, if not quite, entirely anterior to the nucleus.

But from the primitive arrangement of the acrosome, a second series of sperms might possibly be developed by a backward, rather than a forward, extension of the acrosomal material, the anterior end of the nucleus still remaining as a point of reference. Such an arrangement has long been known to be characteristic of the mammalian sperm (fig. 1D), where the amount of acrosomal material is so considerable in respect to the size of the nucleus that it spreads out as a broad sheet over a large part of one side of the flattened nucleus, projecting anteriorly as a broad free edge of the sperm head. It is clear that if the nucleus in such a sperm underwent an elongation similar to that commonly occurring in Amphibia, the acrosomal material would be drawn out along the nucleus in a band, the width of which would be dependent upon the relative abundance of acrosomal material. Such a distribution is, as a matter of fact, common in several groups of insects (fig. 1E), although up to the present time it has received scant attention. I have described the steps in the

production of such an acrosome in the Pentatomidae (Bowen, '22 b), and have been able to follow it well toward maturity. However, with the usual staining methods, it becomes impossible to distinguish it from the nucleus in late stages of spermiogenesis and the relation to the mature sperm head has been in some question. Recently, however, I have found that Cajal's silver method for demonstrating the Golgi apparatus will sometimes impregnate the acrosome intensely and absolutely selectively, the remainder of the sperm being so clear and uncolored as to be differentiated in late stages with difficulty. In such preparations the disposition and extent of the acrosome can be made out with diagrammatic clearness. A series of camera-lucida drawings from a testis of Euschistus euschistoides, in which this reaction was unusually successful, is shown in figure 2.[1] In figure 2A is shown an early spermatid with the Golgi apparatus intensely blackened while the acrosomal vesicle (in the original) is a decided brown and the acrosomal granule a clear red-brown. In figure 2B, the Golgi apparatus is unstained—a rather common reaction in these particular stages. The acrosome, on the other hand, is very clearly stained and may show the usual structures, or it may be so intensely blackened as to obscure all details and lead to its erroneous identification as the Golgi material itself. In figures 2C and D the casting-off and tailward movement of the Golgi mass (Golgi remnant) is shown. In these figures the acrosomal granule is particularly interesting, since by other technical methods I was never able to follow it in these stages. Figure 2D is especially noteworthy as indicating a movement of the granule toward the anterior edge of the acrosome as a whole, which is now moving toward an apical position. In figure 2E the nucleus has begun to elongate, and along one side of it the acrosome is drawn out for nearly two-thirds the length of the nucleus. The anterior part of the acrosome consists of an intensely blackened body which is perhaps the original acrosomal granule, and which can

[1] For a more complete account see Bowen, '22 b, where numerous figures of corresponding stages after more familiar technique will be found.

be similarly demonstrated in Flemming-hematoxylin preparations. The remainder is colored a dark brown. In subsequent stages the impregnation extends to all the material of the acrosome, which is rendered a most brilliant black on the clear canary yellows of the remaining sperm parts (figs. 2F to H). The acrosome can be followed through the various stages in the elongation of the head, retaining its position as a long thread applied laterally to the nucleus beyond which it projects somewhat anteriorly. Posteriorly its relation to the base of the head cannot be so exactly determined due to the indistinct nuclear outlines, but I should say that the acrosomal rod is at least five-sixths as long as the nucleus itself. In stages just before the casting off of the protoplasmic balls from the sperm tails, the acrosome tends to impregnate somewhat unevenly, giving rise to a granular appearance (fig. 2I). In subsequent stages where individual heads are not easily seen in toto by reason of their being so closely packed, the impregnation becomes more uneven and less intense, but it is still visible in cysts very nearly ready to be discharged as mature. These facts leave little room to doubt that the acrosome retains the same lateral position and relative extent in the functioning sperm.

These observations throw an interesting light on the sperms of other insects where the facts are not so well known. Thus, in the beetles Chelymorpha and Lixus I have found a similar nuclear rod which I interpreted as an acrosome (Bowen, '24). In these cases, however, the rod extends to the very base of the sperm head, and when lightly stained may appear as a delicate lateral thread traversing the sperm head from base to apex. From some preparations of Diptera which I have chanced to see, I suspect that an acrosome of essentially this same type may occur in that group. In the cases so far examined the acrosomal rod seems to extend more or less beyond the anterior limit of the nucleus as a delicate apical thread.

I have been at some pains to describe this type of acrosome in detail because it appears to be common among insects

where sperm heads have frequently been measured for the purpose of computing absolute or relative volumes of chromatin. Since these lateral acrosomes may not be distinguishable in the mature sperm, it is clear that errors might be

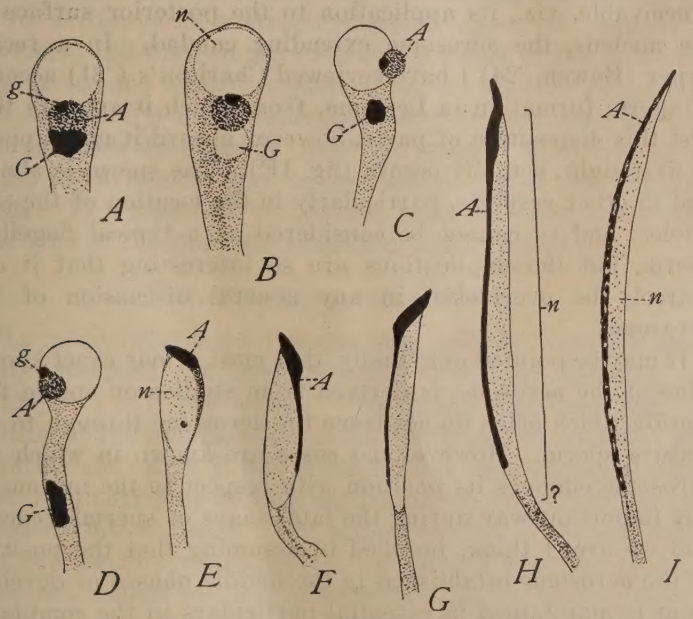

Fig. 2 Stages of sperm formation in Euschistus euschistoides (small-cell generations; Bowen, '22 b); Cajal preparations with the acrosome in later stages impregnated selectively (× 3000). *A*, acrosome; *g*, acrosomal granule; *G*, Golgi apparatus plus idiosome (= Golgi complex or acroblast); *n*, nucleus. (Compare with Bowen, '22 b: *A* with figs. 81 and 103; *B*, with fig. 104; *C*, with fig. 115; *D*, with figs. 117 and 118; *E*, with figs. 136 to 138; *F*, with figs. 143 and 144; *G*, with figs. 147 and 149; *H*, with fig. 157; *I*, with fig. 161 and later).

introduced through disregarding their presence. It would appear that such measurements ought to be undertaken only with some knowledge of the entire spermiogenesis.

It will be clear from the preceding discussion, 1) that the acrosomal material may be attached to the anterior surface of the sperm nucleus and project forward in a great variety of

ways or, 2) that the acrosomal material may be attached along a part or the whole of the lateral aspect of the sperm nucleus. Practically all sperms may be reduced to either one or the other of these types. But a third arrangement of the acrosomal material with respect to the sperm nucleus is conceivable, viz., its application to the posterior surface of the nucleus, the acrosome extending caudad. In a recent paper (Bowen, '24) I have reviewed Charlton's ('21) account of sperm formation in Lepisma, from which it appears that just this disposition of parts, however absurd it may appear at first sight, actually occurs (fig. 1F'). The sperm is abnormal in other respects, particularly in the location of the centrioles, and so cannot be considered as a typical flagellate sperm, but the implications are so interesting that it can scarcely be overlooked in any general discussion of the acrosome.

It may be pointed out, finally, that most of our exact knowledge of the acrosome is derived from studies on sperm formation which often do not trace the acrosome through to the mature sperm. However, no cases are known in which the acrosome changes its position with respect to the nucleus in any important way during the late stages of spermiogenesis, and we are, I think, justified in assuming that the position of the acrosome established in the middle phases of development is maintained in essential particulars in the completed sperm. To this statement the only possible exceptions, so far as I know, seem to be found among the marsupials, in which Fuerst ('87) long ago described the complete casting off of the acrosome (his 'Kappe') in the closing stages of sperm formation. This has been corroborated by v. Korff ('02) in Phalangista, and in some degree supported by Duesberg ('20) in Didelphys. This extraordinary behavior is so inexplicable that further research will be necessary to establish its exact meaning. But it may be pointed out that v. Korff's observations indicate a clear possibility that a part at least of the acrosomal material may remain attached to the head (see his figures 28 a and b). Whether this supposed

loss of the acrosome has any connection with the peculiar copulation in pairs of many marsupial sperms is equally in doubt.

To summarize the mere positional relations of the acrosome in the mature sperm, we may say that the popular conception of the acrosome as a structure purely apical in position is incorrect; and that, on the contrary, the bulk of the acrosome may be located almost anywhere on the nuclear periphery, with, however, a nearly universal tendency for some portion of it to reach the anterior tip of the sperm. With respect to shape, the acrosome may assume either one of two extreme forms: 1) compact or, 2) filiform, between which every possible type of intermediate condition can be found.

THE MEANING OF THE ACROSOME

The meaning of the acrosome, long held and now the commonly accepted text-book statement, is well outlined by Waldeyer ('01), who says: "Its whole structure and its position at the anterior end of the head, as well as direct observation show that it has a mechanical significance, namely to act as a boring or cutting apparatus for the penetration of the sperm into the egg." This interpretation of the acrosome as a cutting tool with a purely mechanical function has for some time seemed to me quite untenable, and in various places I have urged the inadequacy of this explanation. It is unnecessary to labor these points again, and the basis for my abandonment of the 'cutting-tool theory' may be briefly summarized as follows:

1. That the acrosome enables the sperm to cut its way into the egg is a pure inference based largely on the belief that the acrosome is necessarily an apical attachment on the sperm head, and therefore comes first in contact with the egg.

2. The shape of the acrosome, or perhaps better of the sperm head as a whole, may, it is true, enable some sperms to penetrate the jelly coverings of certain eggs more readily than is the case with other sperms of less favorable shape, but the shape seems to play no part whatever when the sperm actually reaches the periphery of the egg.

3. The entrance of the sperm into the egg is accompanied by an active response on the part of the egg at the point where the sperm touches its periphery, and the motionless sperm appears actually to be 'swallowed' by the egg. The entry of the sperm is not attended by any of the mechanical movements of boring or cutting.

4. The shape of the acrosome frequently indicates no adaptation for a mechanical perforator.

5. The disposition of the acrosomal material is frequently such that its major portion could not possibly function as a cutting tool (insects particularly).

6. The old view of the acrosome as a structure built out of any available protoplasmic constituent favored a mechanical interpretation. The new view of the acrosome as invariably developed in association with the Golgi complex points, on the other hand, to some more fundamental purpose.

7. The case of Lepisma appears to be a complete reductio ad absurdum of the 'cutting-tool theory.'

I can see no escape from the conclusion that, while the acrosome may indeed sometimes play a certain mechanical rôle, its real significance is something fundamentally different.

What, then, is the real meaning of the acrosome? I believe that a clue to the answer of this question has been supplied by the remarkably interesting researches of Nassonov ('23, '24) on the origin of secretory granules. According to Nassonov, these granules make their first visible appearance in close association with the strands of the Golgi apparatus, and this relation is maintained for a longer or shorter period as the case may be. The granules which appear as small droplets ultimately become detached and are eventually cast out of the cell in the manner long familiar to students of secretory phenomena. My own observations, while less extensive than those of Nassonov, have convinced me that he is essentially correct in his statements concerning the positional relationship of the secretory granules to the Golgi apparatus. A comparison of his figures with my figures of acrosome formation in various animals reveals a startling

similarity in basic details. If, therefore, our knowledge of the acrosome has made possible an interpretation of the more obscure phenomena in the gland cell (Bowen, '23 b), so, too, these phenomena seem to furnish us with an unexpected possibility for interpreting the acrosome.

In brief, it now seems probable that the acrosome is to be regarded, from its mode of formation, as a secretory product, in the broad sense of the term. Furthermore, the parallel with the gland cell proper may be further extended, the acrosome becoming thus a secretory granule, constructed perhaps from materials related essentially to enzymes.[2] The constant relation of the acrosome to the Golgi apparatus thus becomes explicable and the disposition of the material in the sperm need no longer necessarily be linked in explanation to the mechanical operation of perforating.

If these comparisons be really significant, the function of the acrosome would seem to be connected with the release of this secreted material from the sperm cell. Such a process of 'secretion' is possible at the time of fertilization, when the acrosomal material might be discharged into the protoplasm of the egg. Here again my interpretation fits in with current views of the nature of the fertilization process, particularly as expressed in the views of F. R. Lillie and Just.

We may then summarize[3] these possibilities as follows: *The acrosome is essentially a secretory product the principal function*[4] *of which is to initiate the physicochemical reactions*

[2] In favor of the view here developed, an interesting bit of corroborative evidence has been called to my attention by Doctor van Herwerden. She has shown ('13) by the Roehmann and Spitzer test for oxidizing enzymes that in the sea-urchin sperm (Strongylocentrotus lividus) there is an apical granule (Retzius' ('04, '05) figures of echinoderm sperms indicate that it is the acrosome) which develops a positive reaction indicative of the presence of an oxydone (oxydase). Unfortunately, this interesting observation is somewhat equivocal, since the mitochondrial granules in the middle-piece also respond in the same manner. The possibilities of this line of attack remain, however, most interesting, and stimulate our hopes of developing a test capable of more conclusive results.

[3] See also Bowen, '23 a.

[4] I do not mean by this statement necessarily to exclude the acrosome from participation in other activities of the sperm cells. Thus it has been suggested

of fertilization. This conclusion is not only in accord with certain implications of Lillie's fertilizin theory, but also is in remarkable coincidence with the explanation which Lillie ('12) has considered probable from a morphological study of the penetration phenomena of Nereis. The experiments of Goodrich ('20) on removing the sperm from the Nereis egg very shortly after contact has been established also bear out the supposition that the substance of the acrosome plays an important part in initiating the fertilization reaction.

The problems presented by the acrosome from the standpoint of sperm formation seem thus on the point of a satisfactory solution, and the most pressing point still to be solved is the fate of the acrosome in the egg. A thorough study of this matter might yield either a final confirmation of the views here put forward or a demonstration that the view of the acrosome as a secretory product is untenable.

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that the orientation of the sperms in the testis and their 'nourishment' during the period of differentiation are effected through the acrosome. The evidence in support of these suggestions does not at present seem to me very substantial; but that the acrosome has an interest in these or other directions is not of course impossible.

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THE ANATOMY OF AN OTOCEPHALIC DOG

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EIGHT FIGURES

The specimen to be described in this paper was presented to the Department of Zoology of West Virginia University by Mr. O. N. Riggs, of Morgantown, who supplied the following history:

The mother was a Scotch collie about one and one-half years of age, and the abnormal foetus was one of eight in her first litter, all of the remainder of which were normal and survived.

Prof. A. M. Reese photographed the specimen, then secured the services of our taxidermist, Mr. P. C. Bibbee, who carefully removed the skin, leaving the specimen intact for further study. Doctor Reese, after certain preliminary anatomical studies, kindly presented the specimen to the writer, who had become somewhat familiar with the type of anomaly represented.

DESCRIPTION OF THE SPECIMEN

The intact animal measured 14 cm. crown-rump and was normal externally except for the head. The head was almost round and had two fused ears with no external indication of eyes. While the eyelids were completely absent, a little tuft of coarse hairs just above the fused ears indicated that there may have been the beginning of lash formation at least, although not to the extent shown in the pig described in an earlier communication (Chidester, '14).

This is a typical example of cyclopean otocephaly and may be termed anophthalmia otocephalia. It possessed no mouth or nose and was therefore unable to breathe.

The nasal proboscis, so frequently found above the orbit in cases of cyclopia and otocephalia, was absent. At times one finds the nasal proboscis and a normal nose both present (Wilder, '08).

In rare cases it is found that two probosces occur, one above and one below the eye (Taruffi and Vogt, '94). Allan has described ('48) a human foetus with cyclopean otocephalus and with a suborbital proboscis.

INTERNAL ANATOMY

The specimen was a female with normal reproductive, excretory, digestive, and respiratory systems. The circulatory system was apparently normal except for the defective brain circulation. The thymus, thyroids, and adrenals were normal in position and not larger than those of the control specimen.

SKULL (figs. 1 and 2)

The skull measured 17 mm. diameter laterally, was 16 mm. high, and had an anteroposterior diameter of 16 mm.

On removing the skin from the head the taxidermist necessarily destroyed the relations of the eye-muscle mass, and it was impossible to determine how many of the eye muscles were present. In all probability the obliques and the external recti were present, while, as is usually the case, the internal recti were absent.

Figure 2 illustrates how the premaxillaries were pushed far anteriorly and the maxillaries were spread. The premaxillaries develop independently from the median bud, and the maxillaries arise from the first visceral arch to subsequently unite with the premaxillaries. Wyman ('59) concluded, therefore, that a cyclopean eye, projecting forward in the median line, would have a tendency to throw the pre-

maxillary bud upward and prevent it from uniting with the maxillaries. In anophthalmia otocephalia it is evident that the anterior portion of the skull is much deformed. The nasals were pushed down considerably below the maxillaries

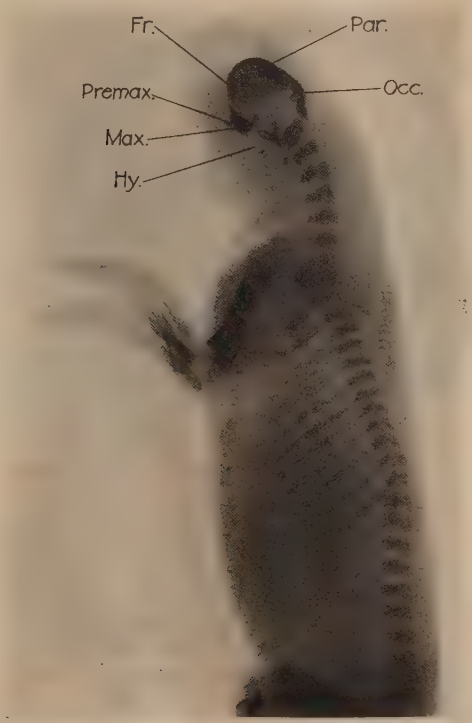

Fig. 1 Skiagraph of otocephalic dog. *Fr.*, frontal; *Premax.*, premaxillary; *Max.*, maxillary; *Par.*, parietal; *Occ.*, occipital; *Hy.*, hyoid.

and were spread, while the mandibles were much reduced in size. The hyoids were approximately normal in position and size. The parietals were considerably reduced in size as were the occipitals, but the frontals were approximately normal in proportion to the size of the animal. As was to be expected, the lachrymals, vomer, ethmoid, and turbinates were

absent. The cause of otocephalus is supposed to be an arrest of the development of the lower processes (Meckel's cartilages) of the first visceral arch. Regarding the primary causes that may be shown to be contributory, we shall conjecture later.

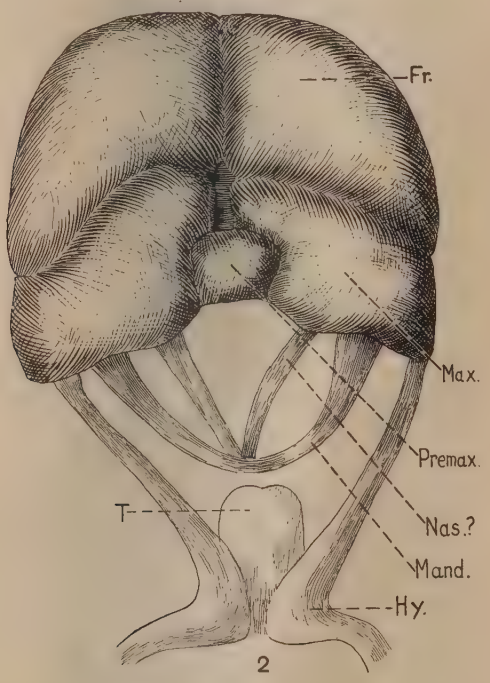

Fig. 2 Front view of the skull of otocephalic dog. × 3. *Fr.*, frontal; *Premax.*, premaxillary; *Max.*, maxillary; *Nas.*, nasal; *Mand.*, mandibular; *Hy.*, hyoid; *T.*, tongue.

INSIDE OF THE SKULL

After studying the brain in situ, it was removed together with about half of the cord and placed in stronger formalin with a control brain, preliminary to sectioning for further study.

On comparison of the abnormal skull with a normal one (figs. 3 and 4), it was discovered that the auditory capsules were placed almost exactly in the center of the cranial cavity, leaving practically no cerebral fossa. While there was a slight

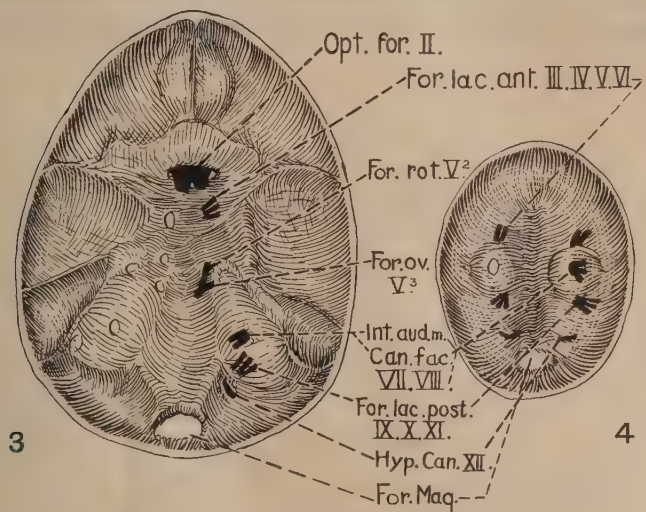

Fig. 3 Inside view of the skull of control specimen. × 1⅓.
Fig. 4 Inside view of the skull of otocephalic dog. × 1⅓.
Opt.for., optic foramen with optic nerves; *For.lac.ant.*, foramen lacerum anterius with oculomotor, trochlearis, and abducens nerves, together with the first branch of the trigeminal nerve; *For.rot.*, foramen rotundum with the second branch of the trigeminal nerve; *For.ov.*, foramen ovale with the third branch of the trigeminal nerve; *Int.aud.m.*, *Can.fac.*, internal auditory meatus with auditory nerve; facial canal with facial nerve; *For.lac.post.*, foramen lacerum posterius with glossopharyngeal, vagus, and spinal accessory nerves; *Hyp.can.*, hypoglossal canal with hypoglossal nerve; *For.mag.*, foramen magnum.

depression in the region that would normally be occupied by the optic chiasma, there was no foramen, nor was there any evidence of an optic nerve. The nerves beginning with the oculomotor (III) and including the hypoglossal (XII) were extremely small and apparently passed through the proper foramina from the normal medulla.

A rather thin dura mater was present, lacking a falx cerebri, but with a quite well-developed falx cerebelli together with a portion of the tentorium cerebelli. The arachnoid and the pia mater were both poorly developed, while the posterior medullary velum was rather thicker than normal.

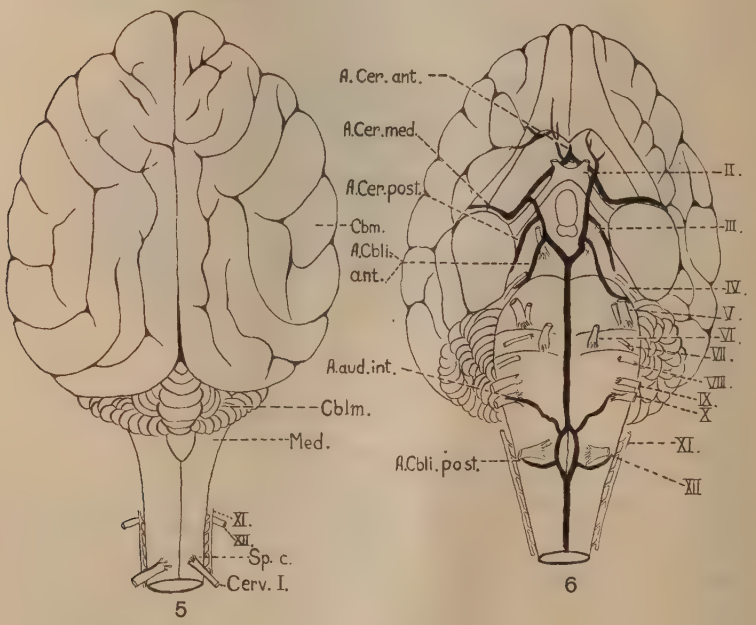

Fig. 5 Dorsal view of the brain of control dog. × 1⅛.
Fig. 6 Ventral view of brain of control. (Scotch collie at term.) × 1⅛.

After the brain and spinal cord had been studied externally and compared with a control specimen, they were divided at the medulla and transverse sections were prepared by a modified paraffin-Weigert method.

The external study of the brain is indicated by figures 7 and 8, together with figures 5 and 6, showing the structure of the control brain used for both gross and microscopic study.

Dorsal view (fig. 7)

The brain was quite small. The cerebellum was 11 mm. in diameter, while the thalamic mass was 8 mm. in diameter and the medulla but 5 mm. in width. There was no indication of hydrocephalus, and in fact the third as well as the lateral ventricles were obliterated. There was complete bilateral sym-

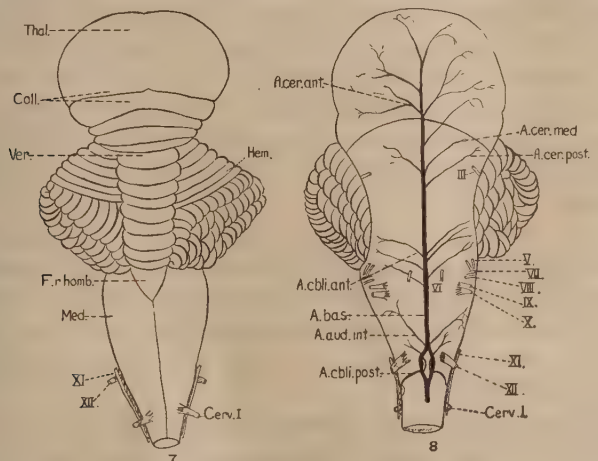

Fig. 7 Dorsal view of the brain of otocephalic dog. ×3½. *Thal.*, optic thalamus; *Coll.*, superior and inferior colliculi; *Ver.*, vermis of cerebellum; *Hem.*, hemispheres of cerebellum; *F.rhomb.*, fossa rhomboidalis; *Med.*, medulla; *XI, XII*, cerebral nerves; *Cerv.I.*, first cervical nerve.

Fig. 8 Ventral view of the brain of otocephalic dog. × 3½. *A.cer.ant.*, anterior cerebral artery; *A.cer.med.*, medial cerebral artery; *A.cer.post.*, posterior cerebral artery; *A.cbli.ant.*, anterior cerebellar artery; *A.bas.*, basilar artery; *A.aud.int.*, internal auditory artery; *A.cbli.post.*, posterior cerebellar artery. *III, IV, V, VI, VII, VIII, IX, X, XI, XII*, cerebral nerves.

metry posterior to the thalamic mass, but the cerebrum and all other structures that would normally have been present as derivatives of the first primitive vesicle were absent. The thalamus was incompletely divided dorsally, and the superior and inferior colliculi were present although laterally compressed. The pineal and pituitary bodies were absent. The cerebellum was smaller than in the control, but was well

formed. The vermis was quite conspicuous as in the specimen described by Duckworth ('08).

Ventral view (fig. 8)

Behind the thalamic mass the pons appeared quite normal as seen on the ventral aspect (fig. 8). The trapezia and pyramidal tracts were, as we might naturally expect, undeveloped. It is worthy of note, however, that Duckworth found ('08), in his study of a microcephalous pig, that the transverse sections of the medulla and cord were similar to those of the control, and concluded that, "this observation seems to support the view that the pyramidal tracts are laid down *in situ,* and owe nothing to fibres descending from the cerebrum."

The nerves beginning with the trigeminal were readily discovered and except for their reduced size were comparable to those of the control (fig. 6). The oculomotor and trochlear nerves were not so easily distinguishable, but it is believed that the nerves indexed as III in figure 8 are correctly designated.

In studying the circle of Willis it was found that, while the vessels were small and in some cases extended only a short distance from their origins, it was possible to trace the anterior, medial, and posterior cerebral arteries, the anterior and posterior cerebellar arteries, and the internal auditory arteries as well as the basilar.

SUMMARY AND CONCLUSIONS

The specimen, a case of anophthalmia otocephalia, had neither mouth nor nose and was unable to breathe. The ears were fused.

It possessed no nasal proboscis and its anomalies were limited to the head.

In common with the majority of otocephalic monsters, the premaxillaries were spread widely and much smaller than normal. The hyoids and the tongue were normal in size and attachment. The frontals, parietals, and occipitals were not

seriously affected. The ethmoid, vomer, lachrymals, and turbinates were undeveloped.

All of the portions of the brain that would normally develop from the first primitive vesicle were absent and the thalamus represented the most anterior development. The corpora quadrigemina were not normally developed and the pineal and pituitary bodies were absent. The cerebral nerves from the fifth posteriorly were small, but apparently normal in arrangement.

In considering the origin of cyclopia we must refer briefly to other anatomical and to striking experimental work that throw light on the subject.

The brilliant experimental work of Dareste and his equally famous countryman, Féré, did much to extend our knowledge of teratology.

Dareste ('77, '80, '91) believed that in the chick, cyclopia was due to the compressing action of the head folds of the amnion which caused the premature closure of the cerebrospinal groove.

Féré ('94,–'03) not only repeated the experiments of Dareste and others on eggs, altering the temperature, causing traumatism, reducing the supply of oxygen, and subjecting them to mechanical vibration, but he tried the effects of gases that penetrated the shell, such as the atmosphere from turpentine, ammonia, ether, chloroform, and tobacco. He also injected substances in solution into the albumen of the egg. He used solutions of salt, glucose, many alcohols, acetone, blood, cocaine, morphine, and atropin. In all of his work, control experiments were carried on and very large quantities of eggs were used.

Féré secured omphalocephaly, atrophy of the head, double heart, atrophy of the tail, cyclopia, and other monstrosities by means of almost all of the injected substances and also by means of subjection to agitation and to gases. While the intensity and duration of action of the disturbance caused a greater number of monsters, Féré did not discover that any specific influence was responsible for a particular monstrosity.

It should be noted that the introduction of anesthetics and toxins into the eggs by Féré was a long step in advance of any previous work in experimental teratogeny. Féré proved that the same anomalies may occur both with and without arrest in the development of the amnion. He believed that a change in nutrition is responsible for the production of monsters.

Born ('97) ligatured salamander eggs obliquely to the first plane of cleavage, securing thereby double heads, one of which was frequently cyclopean.

Lewis, in 1905, produced cyclopean Funduli by pricking the anterior end of the embryonic shield. Mall, in reporting the work ('08), concludes that it is the absence of the tissues that allows them to come together and unite, and that a rudimentary brain is not necessarily a concomitant with cyclopia. It is certainly true that cyclopic abnormality may occur without pronounced anomaly of the brain (see also Lewis, '09).

In 1907, Harrison (Mall, '08) produced cyclopia in frog embryos by the entire removal of the brain.

Stockard discovered ('07) that the eggs of Fundulus heteroclitus developing in a solution of $MgCl_2$ in sea-water developed cyclopic deformities. Later he used alcohol and other anesthetics ('10) as had Féré, and produced a large number of eye defects. In experiments with guinea-pigs reported in 1912, 1913, and 1916, Stockard showed that if the parents are subjected to the fumes of alcohol daily for six days a week, many of the young are defective, some having pronounced eye defects. Apparently the germ cells may be injured, for descendants of treated animals, themselves untreated, may at times become the parents of defective offspring.

Mall ('08), from an extensive study of pathological early human embryos, concluded that cyclopia and other deformities result from the faulty implantation of the ovum, resulting in faulty nutrition.

Child ('11) discovered in 1909 that he could produce cyclopia in the Platyhelminth Planaria by means of anesthetics such as chloroform, alcohols, chloretone, and ether, and it was due to his suggestion that the writer engaged in the use of these substances with Fundulus in the summer of 1909.

McClendon showed conclusively ('12) that cyclopean monsters may be produced in Fundulus by many different substances, such as KCl, NaOH, cane-sugar, and caffeine, thus disproving the earlier beliefs in distinct specificity of substances such as salts and anesthetics.

In some experiments with ether performed in 1909, the writer found that in Fundulus heteroclitus a condition of monopthalmia cyclopica could be induced by ether in one of twin fishes without any apparent effect on the normal development of the other member of the pair (Chidester, '14 a).

In connection with a series of experiments on the influence of extracts from the organs of internal secretion on embryonic development in the rabbit (Chidester, '12) it was shown that heavy dosage of mothers during the first week of pregnancy induced a few cases of anophthalmia and exophthalmos with thymus and thyroid; while if suprarenalin was given in excessive doses, the only apparent effect on the embryos was to increase their growth. The negative findings with suprarenalin indicate the improbability of occasional fright being the cause of a mammalian mother giving birth to deformed offspring. (No one accepts, of course, the ancient theory of maternal impressions producing specific deformities.)

The ancients believed that hybridity was a cause of many anomalies. This notion was, however, successfully combated by Bianchi, who showed in 1741 that when the product of human gestation had a rough resemblance to an animal form, this resemblance might be due to a foetal disease or the product itself might be a mole or retained portion of the placenta. Duval advanced the theory that sterility between animals of different species may be due to an abnormal development sufficient to prevent continued embryonic life after impregnation.

Newman, who secured all types of monsters by the hybridization of fishes ('08, '14, '15, '17), suggested in 1917 that the 'axial gradient' theory of Child, which is a quantitative one, "depending on the rate of metabolic activity," is the true explanation of teratological development in axiate forms.

He suggests that the central nervous system, so highly susceptible in its embryonic development, is an axiate system in itself. He also points out that paralleling the axial gradient there is a "susceptibility gradient, in which the apical end is most susceptible to agents that lower the vitality or retard the rate of metabolism."

Newman's statement regarding cyclopia uncomplicated with otocephalus is general enough to include that associated deformity as it occurs in mammals, and would be phrased as follows. "Suppressed eyes (and ears) either bilateral or unilateral, are the result of inhibition primarily of the apical end of the apico-basal gradient, while eyes too close together or fused in the median line (cyclopia) are the result primarily of inhibition of the median portion of the medio-lateral axis." For a complete statement of Child's own explanation of the theory of axial gradients, see Child, "Individuality in Organisms," Chicago, 1915.

Loeb showed in 1915 that eye defects result in fishes reared in darkness and under low temperature conditions.

Werber ('15) produced cyclopic Funduli by means of butyric acid and acetone, and suggested that monstrous development was due to metabolic toxemia. He emphasized the susceptibility of the anterior end to agents.

Kellicott induced abnormal development including cyclopic deformities ('16) in the fertilized eggs of Fundulus heteroclitus by means of low temperature. He concluded that the cause of the abnormal embryonic development was a disturbance of the normal organization of the ovum, suggesting his 'disorganization hypothesis' as a substitute for the nutrition and metabolism hypothesis of Mall, Stockard, Werber, and Loeb.

Gladstone and Wakely ('20, '23) incline also to the belief that cyclopia and agnathia, like hare-lip and cleft palate, are attributable to defective nutrition at the proper stages.

Whether there may not also be a germinal factor in some cases of cyclopia and otocephalus is not yet clearly shown. Wilder ('08) believes that there is such a germinal factor.

Certainly, the case recorded by Brotherston (Symington and Woodhead, '88) of a cyclopean lamb which was one of thirty similarly deformed, all the offspring of one ram, is hard to explain otherwise. Also the results of Stockard with guinea-pigs ('12, '13, '16) prove that alcohol may affect the germ cells of the male as well as the female offspring of a treated mother.

In a recent study of otocephalus in the guinea-pig, Wright and Eaton ('23) have emphasized the importance of an inquiry into the possibility of the action of specific unit factors. They conclude that development in a given cell lineage "is a chain reaction in which each gene reacts only in the presence of certain conditions, in part environmental relative to the cell lineage in question, and in part the result of the action of genes previously called into action. In otocephaly, specific unit factors have not been demonstrated, but their presence is probably indicated by the evidences of segregation in early generations and the sudden jumps in percentage of monsters at points in particular inbred lines."

The authors conclude that the apparent loss of determinants for parts of the head may be explained as due to genetic factors with a very simple physiological effect, acting in conjunction with environmental conditions.

Out of 40,000 guinea-pigs recorded in genetical experiments, eighty-two otocephalic monsters appeared. The majority were in one inbred family, in which the frequency was 1.5 per cent.

Wright and Eaton are inclined to minimize the importance of environment and the physiological condition of the mother.

In opposition to this theory, evidence is of course presented by cases of cyclopia in one of identical twins in man and the mammals, in addition to the experimental case already mentioned by the writer ('14 a).

The best evidence available would seem to show that in mammals disturbances of the metabolism of the mother, due to agents affecting digestion and the hormonic equilibrium, so change the blood as to cause nutritional defects, such as cyclopia and otocephalus.

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REGENERATION OF STRATIFIED SQUAMOUS EPITHELIUM

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TWO PLATES (EIGHT FIGURES)

The method of replacement, or regeneration of epithelia has held the attention of investigators since the formulation of the cell doctrine. The first attempts were in the direction of establishing the method of cell division rather than the study of regeneration in any particular tissue. Later, with the establishment of details pertaining to direct and indirect cell division, attention was diverted to the study of the processes involved in the regeneration of the various tissue groups. The literature on the subject, especially that restricted to the study of regeneration of stratified squamous epithelium, is not very extensive.

Walther Flemming(1) in 1879 noted the occurrence of cells containing mitotic figures in the deeper strata of the skin of larval and adult salamanders. In another article a year later Flemming(2) came to the definite conclusion that "The regeneration of epithelia as well as other tissue cells occurs through cell divisions in the deeper strata accompanied by the now familiar phenomena called karyokinesis." Pfitzner(3), in an article on "Further observation on Karyokinesis," in 1882, briefly mentions again the appearance of mitotic figures in the lower strata of larval and adult salamanders. Evidently his preparations were the ones to which Flemming had reference in the above work. Flemming(5), continuing his studies on regeneration, paid particular attention to the

regeneration of epidermis in mammalia, using for his material the snout of the pig. He found quite a number of mitotic figures in the deepest layers of the stratum germinativum and remarked that "In the large-celled epithelial masses with broad intercellular spaces, peripheral to the deeper strata and constituting the main mass or the chief epithelial masses, no mitotic figures were ever found; mitotic figures were constantly contained only in the stratum immediately adjacent to the connective tissue of the papillae."

Unna(4) calls attention to the occurrence of mitotic figures in the stratum germinativum of the skin, but expressed doubt as to whether they represented normal processes, since they were in the neighborhood of condylomatous areas.

In a further article on the same subject, Flemming(6) mainly refers to the work on stratified squamous epithelium previously done by him and others.

A survey of the various text-books of histology, including American, English, German, and French authorities, gives no additional information, proving, however, that the authors hold very similar views pertaining to the mode of reproduction of stratified squamous epithelium. Without one dissenting voice they are in accord that the stratum germinativum is responsible for the production of the new cells. With the exception of the two French text-books (12, 24) consulted, they further agree that the stratum germinativum consists of the stratum cylindricum and the stratum spinosum, the former being the single basal layer of columnar cells, the latter, composed of several layers of large polygonal cells, separated by prominent intercellular spaces and connected with each other by intercellular bridges—the 'Tonofibrillen' of M. Heidenhain.

The two French text-books consulted are very specific in designating the single basal layer of cells as the germinal layer, couche germinative; however, they admit that cell division is not limited to this stratum, but occurs also in the polyhedral cells of the next layer—the stratum spinosum.

The greatest difference of opinion encountered is in regard to the localization of the particular portion of the stratum germinativum which continues actively in the reproduction of new cells.

The various views expressed were held sufficient cause to warrant an analysis of this question and to reopen the problem of regeneration of stratified squamous epithelium to further investigation.

No doubt there are many reasons why the opinions were not uniform. A study of the works consulted reveals the difficulties encountered by the pioneers of histology. The process of cell division itself was something new and not yet thoroughly understood in its various phases. Histological technique, then still in its developmental stages, did not yield the accurate results which may now be obtained. Lastly, it may be said that when observations were made that tended to disprove something already established, such observations were often attributed to pathologic changes in the tissues.

Classifying the various opinions relative to the source of new cell production, as outlined in the text-books consulted, they may be divided into the following groups:

I. Those who consider only the basal layer of columnar cells actively engaged in the production of all new cells(13, 14, 23).

II. Those who designate the basal layer plus the one or two layers of polygonal cells immediately adjacent (10, 12, 18, 21).

III. Those who simply specify the lowest layers of the stratum germinativum(7, 11, 15, 17, 19, 22).

Notwithstanding the diversity of opinion relative to the localization of active cell proliferation in the stratum germinativum, a summary of the present views pertaining to the mode of cell reproduction and differentiation of stratified squamous epithelium may be expressed as follows:

The cells arising from the stratum germinativum are pushed peripherally, becoming differentiated and assuming in turn the characteristics of the cells of the particular layer which they occupy.

It may not be amiss to point out that not in a single instance has attention been called to the appearance of mitotic figures in the central or peripheral portions of the stratum spinosum, hence the following observations should be of interest to the histologist and pathologist.

The question of the various views which pertain to the keratinization of the squamous cells does not concern us here and will be omitted.

MATERIAL

For this study serial sections from the scalps of several different individuals were used.

The material was fixed in Bouin's fluid, Zenker's fluid, or formalin. Among these tissues were some which I received through kindness of Dr. Irving Hardesty, of Tulane. They were obtained from autopsy following execution. Blocks of these tissues were embedded by standard methods in paraffin and celloidin; sections of the various organs proved to be not only normal, but were exceptionally beautiful. The celloidin sections were cut at 10 μ and stained with alum hematoxylin and eosin.

OCCURRENCE OF MITOTIC FIGURES IN THE STRATUM GERMINATIVUM

Cytologic details throughout the sections, especially in the sebacious glands, the hair follicles, and the stratified squamous epithelium, were unsurpassed. A careful study of the sections yielded results which are at variance with the accepted views concerning the mode of regeneration of stratified squamous epithelium.

The stratum cylindricum is generally described as a single layer of soft, columnar cells, having no cell membrane, and resting directly upon the basement membrane which separates the epithelium from the underlying connective tissue. (The numerous papillae which rarely permit views of these cells perpendicular to the basement membrane have no doubt made observation of these cells difficult.) The cells of this layer appear quite as individual as those of the stratum

spinosum, possessing not only a cell membrane, but also intercellular fibrillae. On their basal surface delicate protoplasmic processes may be observed which fit into corresponding depressions on the upper surface of the basement membrane.

The number of mitotic figures encountered in the stratum cylindricum was surprisingly small. Without doubt, in the development of stratified squamous epithelium in the embryo, and again during regeneration after injury, this basal cell layer plays the principal rôle in the production of new cells. However, it appears that in the fully formed epithelial tissue this layer serves to maintain the integrity of the epithelial tissue rather than to reproduce new cells per se which subsequently are destined to become components of the superficial layers. This supposition is now the object of further investigation and will be reported at some future date. Suffice it to state that there is evidence on hand from the study of epithelial tumors which seems to warrant the above assumption.

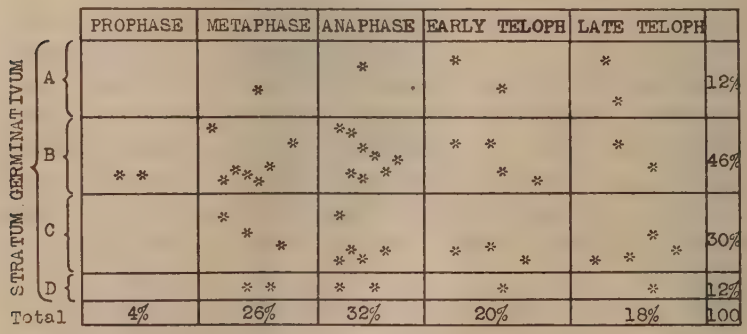
The question of the actual location of the actively proliferating portion of the stratum germinativum, which heretofore has given rise to quite divergent views as expressed in the classification of the three groups of text-books cited, may be answered by stating that cells in mitosis were encountered in all parts of the stratum spinosum except in one or two of the most peripheral rows of cells. The cells of the latter were assuming characteristics of the cells of the stratum granulosum, i.e., granular cytoplasm, poorly staining nuclei, and a tendency to flatten parallel to the surface of the skin.

A quantitative study of the number of mitotic figures encountered gave the results recorded in table 1.

Fifty sections of each from five different scalps were used, each one being 10 mm. in length and 10 μ in thickness, representing a surface area of 25 sq.mm. of scalp.

Since the cell count including the distribution of the cells was quite uniform, the table shows the average per fifty sections or 5 sq.mm. of scalp.

A study of this table shows that if the rete Malpighi were divided into an inner, a middle, and an outer third, exclusive of the basal layer of cylindrical cells, we would find 30 per cent of all mitotic figures contained in the inner one-third, 46 per cent in the middle one-third, and 12 per cent in the outer one-third. In the single basal layer the remaining 12 per cent are found. If we now add the number of mitotic figures found both in the basal layer and the inner one-third, which would safely include not only one or two layers immediately



A.B.C., outer, middle, and inner third of stratum spinosum.
D., stratum cylindricum.
*, approximate position of mitotic figures encountered.

adjacent to the basal layer, but all the deepest layers of the stratum germinativum, we shall have accounted for only 42 per cent of all the mitotic figures found. These 42 per cent represent the three groups of opinions outlined on page 33 relative to the actual locality of new cell reproduction. The remaining 58 per cent, or a majority, were located more peripherally to those.

Since practically all text-books specify the deeper layers of the stratum germinativum as being wholly responsible for the production of new cells, the above figures are in great contrast with the accepted views. The single basal-cell layer, the stratum cylindricum, and the stratum spinosum together should be regarded as the stratum germinativum of the strati-

fied squamous epithelium, at least in the scalp. With the accumulation of eleidin granules in the outermost cells of the stratum spinosum, giving rise to the stratum granulosum, and the disintegration of the chromatin material of their nuclei, these cells lose the faculty to reproduce.

A total of 250 cells in mitosis were encountered, which made an average of ten to every square millimeter of scalp. No mitotic figures were observed near hair follicles, probably due to the obliquity of the plane of section to the cells in that region.

SUMMARY

1. The term stratum germinativum should include the single basal layer of cells, the stratum cylindricum, and the entire stratum spinosum; in other words, the rete Malpighi or rete mucosum.

2. New cells are not only reproduced in the lower layers of the stratum germinativum, but throughout the entire stratum spinosum. The proportion of mitotic figures, in fact, is greater in the middle and outer one-third than in the deeper layers.

3. The cells of the stratum cylindricum which are usually described as having no cell membrane, in the sections above observed, proved to be discrete cells having cell membranes and intercellular fibrillae.

4. Proof that the entire stratum spinosum is actively engaged in cell reproduction will no doubt throw new light on the problem of epithelial tumors.

5. There was no evidence of any coexisting pathology in the tissues studied.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

Unretouched microphotographs, × 300.

Sections of human scalp

1 Metaphase in lower third of stratum spinosum.
2 Anaphase and late telophase in lower third.
3 Metaphase approaching outer part of lower third.
4 Late telophase in central portion of middle third.

Stratum cylindricum showing discrete cells with intercellular fibrillae visible in all four microphotographs.

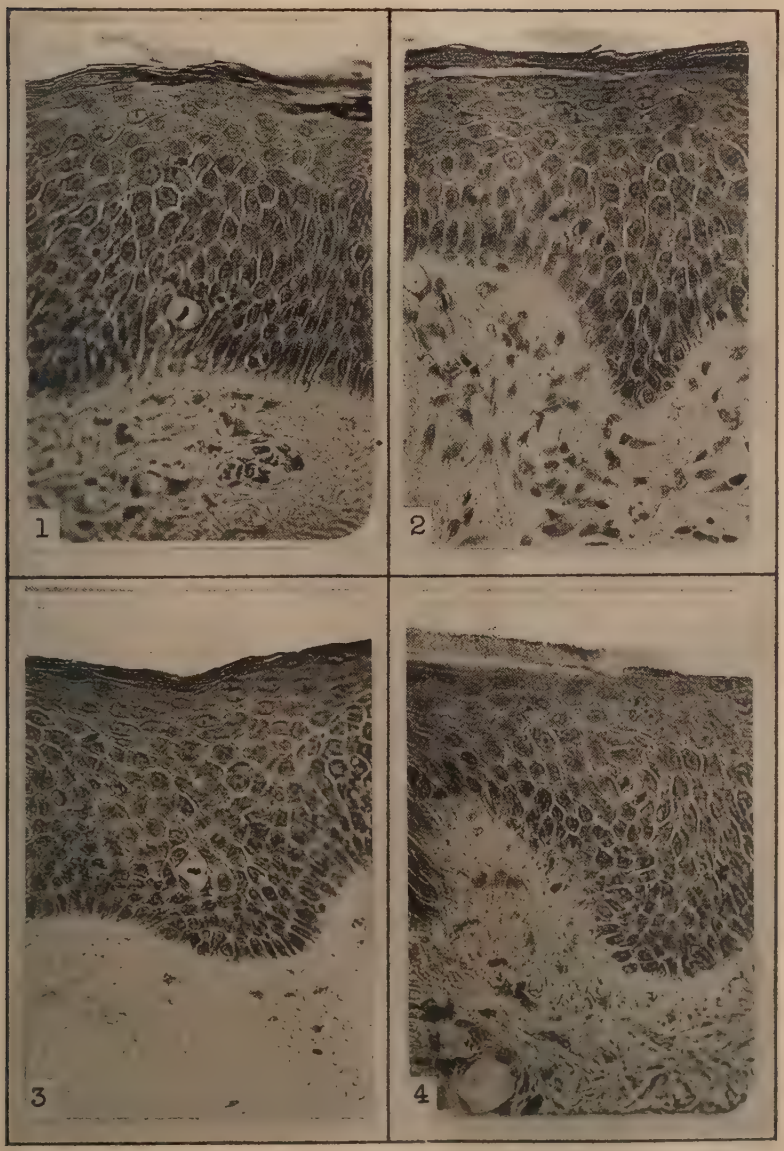
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PLATE 2

EXPLANATION OF FIGURES

Unretouched microphotographs, × 300

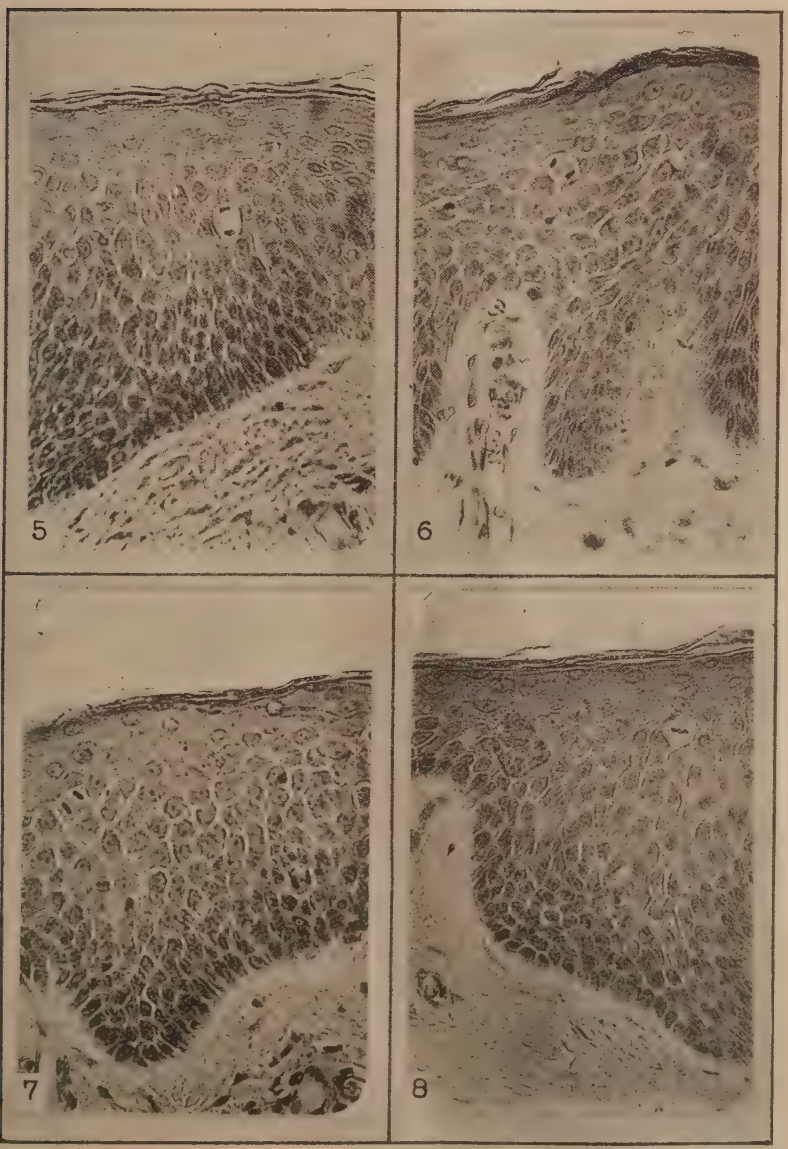
Sections of human scalp

5 Anaphase in middle third of stratum spinosum.

6 Anaphase in peripheral portion of outer third adjacent to cells of stratum granulosum.

7 Late telophase in outer third, early telophase in lower stratum spinosum.

8 Metaphase in peripheral portion of outer third of stratum spinosum adjacent to cells of stratum granulosum.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THE PULSE OF GROWTH IN MAN. A PRELIMINARY REPORT

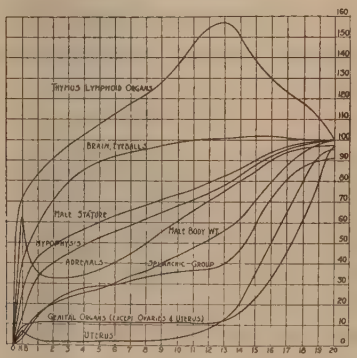
R. BENNETT BEAN
Laboratory of Anatomy, University of Virginia

SIX CHARTS

The alternation of slow and rapid growth of the body has been recognized by those who have studied growth in children, and periods of 'stretching' and 'filling' have been determined at different ages by various observers. Godin showed by a series of measurements of the same children over a period of years that adjacent long bones alternate in their growth in both length and breadth. The bones enlarge in diameter for six months and elongate for the next six months. The periods of activity and repose which succeed each other are opposite for the two serial bones of the same extremity. The lower extremities grow rapidly before puberty, the trunk after puberty, which makes the increase in weight chiefly osseous before puberty, and it is chiefly muscular after puberty. The chief growth in stature occurs during the year preceding puberty; the chief increase in weight occurs during the two years of puberty and the year following.

Puberty seems to be the key to postnatal growth in a general way, because the activities of the cells in growth after the onset of puberty are similar to those at the time of conception, and the curves of growth are similar for the two periods. The period of puberty is the period of maturation of the organs of reproduction, and also of the brain, but it is only the beginning of the last step in the maturation of the other tissues of the body and particularly of the muscles, which grow more after this time. Pigmentation changes

occur at this time, as evidenced by the darkening of the hair and the lightening of the iris. Blood pressure increases at puberty, at the same time that the adrenals grow rapidly in size. The most characteristic alterations that take place in the body are those that relate to the three primary germ layers of the embryo. The organs derived from the entoderm,



Charts 1 and 2 give the actual size at each age, whereas charts 3 and 4 give the percentage increment. The latter method shows better the alternation of slow and rapid growth, and it was largely by the use of this method that the pulse of growth was determined as given in chart 5.

Chart 1 Adapted from Scammon as given in Morris' Human Anatomy with the addition of male stature and male body weight. The curve for the growth of the stature and for the hypophysis are nearly parallel, whereas the curve for body weight is more or less a composite of the other curves. The size of the organ is based on the size at 20 years which is represented by 100. N.B. is newborn; 1–20 is years; 0–60 is percentage of adult size.

as the thymus for instance, begin to involute (chart 1), whereas the organs derived from the ectoderm are arrested in their evolution, and the mesodermal organs, such as the muscles, increase in size and continue their evolution. This is especially true of the gonads and adrenal bodies.

The continuity of the germ plasm has a number of phases that are of considerable interest, according to Godin.

Phase 1. Embryo-fetal. The germ searches its way, then birth ensues.

Phase 2. Childhood. The germ has found its way, then it sleeps.

Phase 3. Youth and adolescence. The germ awakes and matures during puberty, and the soma matures during adolescence.

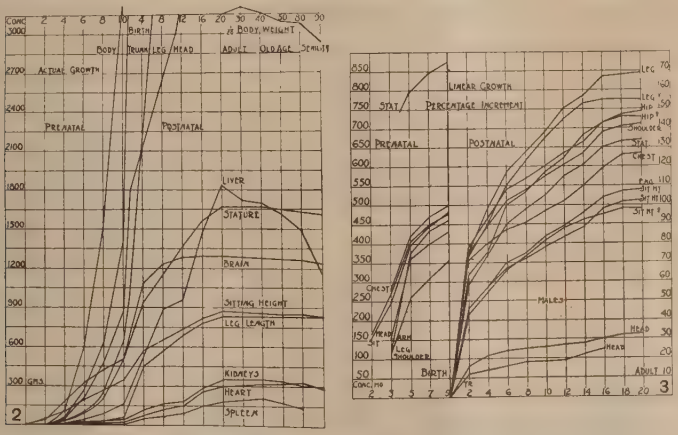


Chart 2 Actual size, prenatal and postnatal. The top row of figures indicates months from conception to birth and years from birth to senility. The linear dimensions which are in centimeters are not directly comparable with the other dimensions which are in grams. The line representing body weight is broken and left out between birth and 20 years.

There is a sharp increase in actual size with all the measurements after birth. The increase is greater for the brain and liver than for the heart, spleen, and kidneys. The trunk, leg, and head volume increase more rapidly than any of these. The stature increases more rapidly than the leg length and the latter more rapidly than the sitting height.

The liver, spleen, heart, and kidneys have a slow increase from 8 to 12 years, which becomes rapid from 12 to 16, decreasing then to 20. After this the liver decreases in size rapidly to the age of 30, more slowly to 40, and again rapidly with rising increment to 90 years. The heart increases slowly to 80, then decreases rapidly to 90 years. The spleen increases slowly to 40 and then decreases rapidly to 80. The kidneys remain the same from 20 to 40, then decrease slowly to 90.

Chart 3 To illustrate the variations in growth of various parts of the body at different times. The prenatal records are from Michaelis, and the postnatal from Weissenberg, West, Boas, Bean, and others. The records are for males except where the female sign ♀ is given.

Growth is rapid from birth to 2 years, less rapid to 6 years, at which time there is a decided slowing. There is an increase in rate of growth from 14 to 16 years in nearly all the measurements. This comes earlier in girls than in boys, and is less marked in the former.

Phase 4. Adult. Plentitude of reproduction of the germ.

Phase 5. Old age. Continuity of the germ in the descendants.

The periods of life may be divided as follows:

Prenatal: 1. Ovum. The first two weeks of pregnancy.

2. Embryo. The next six weeks.

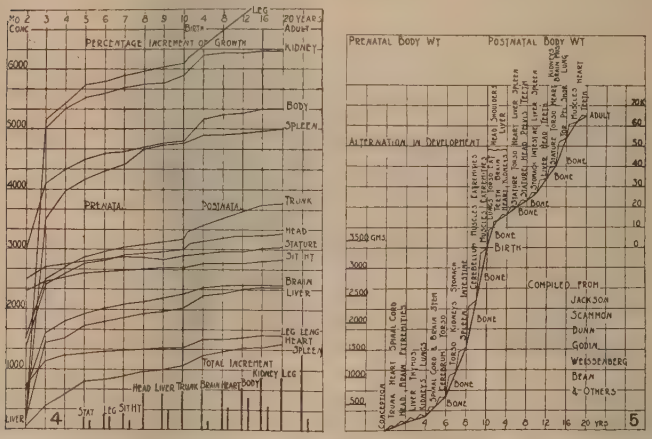


Chart 4 The percentage increment of growth. The percentage increment for each age is based upon the size at the beginning of the age period. All increments are comparable. The increments for the first and second months are so great that they could not be put on the chart. The increment for the third month is greater than for the whole subsequent period except of the heart, trunk, body, sitting height, and stature. At the bottom of the page the vertical lines represent total increments, those on the left are for the last seven months of prenatal life, and those on the right are for the 20 years postnatal. The prenatal increment for the spleen is great because it has grown so little in the first two months, and the same is true for the kidneys and leg volume. The growth after birth is small for these parts. The body, heart, and brain have almost the same increment before birth, but the body increment is more and the brain less than the heart after birth. The liver, heart, and torso increment is half as much after birth as before. The leg-length increment is almost as much after birth as before, but the stature is less. The percentage increment of leg volume is greatly in excess of the increment of leg length both prenatal and postnatal, as is also the body volume greater than stature.

Chart 5 To illustrate the pulse of growth. The smooth solid line gives the size of the body at each age. The approximate period of rapid growth for both prenatal and postnatal periods is shown by the lettering.

3. Fetus. The last eight lunar months of pregnancy.

Postnatal: 1. Newborn. The first two weeks after birth.

2. Infancy. Until the end of the first year. Assumption of erect posture.

3. Childhood.

a. One to six years—milk-tooth.

b. Six to ten years, middle childhood—permanent tooth.

c. Ten to twelve years—prepuberal.

4. Puberty. For two years, beginning at 12 to 17 years.

5. Adolescence. Three years after puberty.

6. Preadult. The three years following adolescence.

7. Adult. May include preadult. From 20 to 100 years or later.

a. Prime, 20 to 40 years.

b. Transition, 40 to 60 years.

c. Old age, 60 to 80 years.

d. Senescence, 80 years and over.

Only the prenatal and postnatal periods as a whole will be separated. Shultz has recently, in some unpublished work which it has been my privilege to examine, shown that nearly all parts of the body enter into the pulse of growth in the prenatal period. Jackson worked on the most important organs during this period and showed that they have maxima and minima of rapid growth. Michaelis has a few linear measurements also for the prenatal period (charts 3 and 5). Scammon has made curves of growth which are smooth and show only the large differences in rate of growth postnatal (chart 1).

Growth is not a straight-line affair nor is it a smooth curve, but it increases by steps, sometimes by leaps and bounds; at times it is slow, then again it is rapid. This is true not only for the body as a whole, but also for its parts or organs, or functional systems; and even parts of organs have a different rate of growth at different times. The cells themselves do not all divide and multiply at the same time, but first one then another becomes active, and those that divide consecutively are not usually the adjacent ones.

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Bardeen first called attention to the 'critical period' in the development of parts of the body, and Stockard limited this to the first part of the 'critical period' which he called the 'critical point.' This is the initial stage of the rapid growth, and influences at this time are more potent.

Anyone who has demonstrated chick or pig embryos at various stages of development to classes in embryology could hardly have failed to be impressed by the difference in relative size of the organs at different stages in development. Adjacent structures, such as the head and trunk, heart and lungs, liver and intestine, seem to be complementary in development because their periods of rapid growth alternate. There may be other parts of the body at some distance from each other that also alternate. The shifting of the locus of rapid growth is of unknown origin, although Jordan and Speidel have shifted the locus of blood formation in the frog earlier than normal by feeding thyroid, and it may be that some such factor shifts the locus of activity in growth from one part of the body to another.

No two adjacent teeth break through the gums consecutively, except the lower canines and lower median premolars, the order of eruption of the permanent teeth being: lower first molars, lower median incisors, upper first molars, upper median incisors, lower lateral incisors, upper lateral incisors, upper median premolars, lower canines, lower median premolars, upper lateral premolars, upper canines, lower lateral premolars, lower second molars, upper second molars, lower third molars, and upper third molars. The teeth appear in two series, the molars form one and the remaining teeth the other. There are four periods of rapid growth of the teeth, the first from 1 to 2 years, when the temporary teeth are coming through; the second about 7 years, when the molars and incisors of the permanent set are erupting; the third about 11 years, when the second molars appear with the canines and premolars, and the fourth when the wisdom-teeth come through, which is about 18 years or later. When the two series are taken separately the molars appear in regular order, but the other teeth do not, except the incisors.

The periods of rapid growth in stature represent bone growth, and they alternate with the periods of rapid growth of the teeth, the first being immediately after birth, the second at 4 years, the third at 8, and the fourth at 16. The prenatal periods of most rapid growth in stature are during the first month when the somites are developing rapidly, during the fifth and sixth months following a rapid growth of the spinal cord and brain stem, and immediately before birth, associated with great motor activity of the fetus and following the rapid growth of the cerebellum and muscles.

The parts of the stature are not equal or coincident in growth.

The torso grows rapidly during the first month of prenatal life with the rapid development of the heart and somites, and during the sixth and seventh months following the rapid development of the cord and brain stem, coincident with the rapid growth of the viscera. The postnatal growth is great during the first year after birth, at which time the lungs, liver, brain, and head grow rapidly in conjunction with the activities of respiration and alimentation which are taken over by the child from the mother at birth. The second period of rapid postnatal growth occurs at six and seven years, when the heart, liver, spleen, head, and pelvis are growing rapidly. The third and final period of rapid growth of the torso is from 13 to 16 years in connection with puberty and its varied manifestations, at which time the body broadens, the pelvis enlarges, the brain, kidneys, and lungs grow rapidly and the muscles develop more than at any other time in life.

The trunk follows the torso in its periods of rapid growth, but the head growth depends upon the growth of its constituent parts, the brain and the face. The head grows rapidly in the second month of prenatal life when the brain is unfolding, after which its rate of growth diminishes more and more until it drops suddenly in the last month before birth. The head grows rapidly after birth during the first few years with the rapid growth of the brain and the growth of the teeth and jaws, again from 6 to 10 years when the permanent teeth are

developing rapidly, and later from 11 to 14 years when the bones of the head are getting thicker, the teeth are developing rapidly, and the air sinuses about the nose are expanding.

The two regions of the head, the cranium and face, grow in their parts unequally. The parietal part of the cranium grows most rapidly about 5 years, the occipital region about 10 years, and the frontal region about 15 years. The length of the cranium grows more than the breadth partly by a thickening of the bones in the occipital and frontal regions and a thinning in the temporal region, partly by enlargement of the frontal sinuses, and partly by a thickening of the fascia over the forehead and occiput. The lower part of the face, including the jaws and teeth, grows more before 12 years and the upper part including the nose and air chambers grows more after 12 years.

The lower extremities grow more rapidly than the upper throughout the period before birth, except for the first month, and this is especially marked during the last month. After birth rapid growth continues for the first year and occurs again at about 4, 8, and 12 years, at which periods the upper extremities are also growing rapidly, but not so rapidly as the lower. The parts of the extremities alternate in growth each six months. The long bones that grow rapidly in length for six months grow slowly for the next six months, but become thicker, and this different kind of growth alternates for the long bones in adjacent segments of the limbs. For instance, if the humerus grows rapidly in length from January to July, the radius and ulna grow rapidly in length from July to January, and at the time that the humerus is growing rapidly in length the radius and ulna are growing rapidly in thickness, and vice versa. There is thus seen to be not only a seasonal variation in growth, but a semestral variation in different parts of the same structure.

Scammon has shown that the splanchnic group of organs have a very rapid growth during the first year of life (chart 1), a gradual slowing to the age of 6 years, then a gradual increase up to 12 years, at which time the growth

becomes accelerated until between 14 and 16 it is very rapid, to slow down again until the age of 20 years is reached. These organs have been studied in detail from 1430 records of the Touro Infirmary and Charity Hospital, New Orleans, and the Johns Hopkins Hospital, Baltimore, and by using the percentage increment method basing the growth on the size at the beginning of each month before birth and the size at the beginning of each year after birth, it has been found that there is also a period of rapid growth between 2 and 8 years. The growth of the heart is slower during the first two years than is that of the liver, spleen, or kidneys, but after that time until maturity is reached the rapidity of its growth is greater than that of the other organs. The heart develops during prenatal life most rapidly during the first month, and afterward it generally keeps pace with the body in its development. There is a relative decrease in the increment of growth in each period from the first prenatal to the last postnatal.

Heart weight, size of person, and physical activity are synchronous. For the first few months after birth the child is not very active, and during this period the growth of the heart is slow. From 6 to 9 months of age the child becomes more active, the growth of stature increases, and the heart enlarges rapidly. Then follows a less active period, when the child is learning to stand and walk, and during this time the heart is relatively inactive. After the child has learned to walk and run, a period of great activity ensues and the stature and heart weight increase rapidly. This is about the age of three or four years. The heart has a slight increase in rate of growth again at five or six years with a rapid increase in size of the alimentary organs. The next period of rapid growth is about the time of puberty, which is earlier in girls than in boys. All the parts of the body reach their rapid growth earlier in the former, and at this time bone growth is particularly active. The final period of rapid heart growth is from 16 to 20 years, when the muscles of the body are undergoing their most vigorous growth.

The most active period of prenatal growth of the liver is during the third month, and after birth the liver weighs about the same for two months, when the first period of postnatal increase in growth sets in to last for a few months. There is another period at 3 years, another at 5 years, and still another at about 10 years. There is a decrease in the increment from the first to the last period. The liver is relatively large at birth and the pelvis is small, and a great part of the abdominal protuberance at this time and for some time afterward is the result of the large size of the liver, as well as the small size of the pelvis.

Before birth the kidneys function little if at all, and their growth is very slight except during the fifth and seventh months. The initial increase in growth rate which comes from 3 to 12 months after birth is greater and more rapid in the kidneys than in other splanchnic viscera, and their maturity seems to be reached earlier, at about 15 years. Their growth is slow after the first year until four years after birth, when they grow rapidly at this time of great physical and mental activity, increased alimentation, and the consequent greater necessity for the elimination of waste products. Again, there is a very rapid growth during puberty with the coincident rapid growth of the skeleton and heart which precedes that tremendous expression of sexual, motor, mental, digestive, circulatory, and respiratory surge that comes between 16 and 20 years.

The most active period of growth in the spleen before birth is during the seventh month, and after birth for a while in the second six months of the first year and about 4 to 6 and 8 to 10. The spleen is more irregular in size than any other organ of the body and its periods of rapid growth are not so clear-cut.

The lungs grow most rapidly before birth in the fourth month, and after birth during the first and from the fourteenth to the sixteenth years. The time of rapid growth is coincident with the rapid growth of the torso, and of the heart and kidneys, and represents the periods of most active oxidation.

The alimentary canal grows most rapidly during the seventh and eighth months before birth and during the ninth and tenth years after birth. The liver and alimentary canal alternate in periods of rapid growth. The stomach grows more than the intestine early in fetal life, but the intestine grows more later, until at birth it is six times the size of the stomach.

The muscles grow most rapidly immediately before birth and immediately before the adult state is reached, both periods of great motor activity.

The spinal cord precedes the brain in its periods of rapid growth before birth. The spinal cord grows most rapidly during the first and fifth months of prenatal life and the brain during the second and sixth months, and the cerebellum grows most rapidly in the ninth month. After birth the brain grows most rapidly during the first four years and again there is a slight rise in the rapidity of growth from 12 to 16 years.

The sex glands, adrenals, and thymus grow rapidly during the first few months of the prenatal period, and the spleen, thyroid, and thymus grow rapidly during the last few months. The thymus and lymphoid organs grow rapidly during the first few years after birth and also from 10 to 12 years, after which they decrease in actual size until the adult state is reached.

The hypophysis and stature have a parallel growth. The adrenals and uterus decrease in size immediately after birth, and the adrenals and sex organs increase in size rapidly at a later period, the adrenals at 8 to 16 and the sex organs at 10 to 14. The adrenals seem to have a direct relation to sex in activating the sex glands and also sometimes later in life in altering the secondary sexual characteristics.

The time at which the organs begin to decrease in weight is of interest from the standpoint of death rate and as an indicator of age. The thymus and lymphoid organs begin their involution at about 12 years of age, which is the age of the lowest death rate after birth. The death rate decreases from birth to 12 years and increases thereafter. The next organ to undergo involution is the liver, which begins at about 20 years of age. The amount of involution in these organs is

greater than in any others. The brain weight begins to decrease about the age of 16 years, but this decrease is so slight until the age of 80 that it may not be significant. The whole body weight begins to go down at 30 years, and Todd and Ingalls have found that bones decrease in weight from about this time. The kidneys and spleen begin to decrease in weight at 40 years, and the decrease is greater in the spleen than in the kidneys. The stature also begins to decrease at 40, although the torso shows a slight decrease from 20 years onward. The heart increases in size up to 80 years, after which it decreases rapidly. The diseases to which the heart is liable and the hardening of the arteries with advancing age increase the work of the heart, which may account for its continued increase in size, therefore this may not be an exact old-age indicator.

Minot calls old age largely a loss of cell fluids, therefore the decrease in size should mean advancing age, but the heart overcomes this by its powers of recuperation, or of growth.

SUMMARY

Growth in man is much more rapid before birth than afterward. The total increment in volume from conception to maturity is about 130 billion times, and the increment before birth is about 6.5 billion times, whereas that after birth is only twenty-fold. On the other hand, the body acquires but 5 per cent of its adult weight before birth and 95 per cent thereafter.

Growth in length is similar to growth in volume or in weight, although relatively less. The total increment from conception to maturity is about 17,000 times, the increment before birth is 336 times, whereas the increment after birth is only about 3.3 times.

Growth is almost infinitely more rapid during the first month than it is the last month before birth, and it is greater by far during the first year after birth than during the last year before maturity. The ripe human ovum has a volume of approximately 0.000,000,5 cc. This increases 8000 times

during the first month of prenatal life. If growth continued at the same rate until it ceased in the adult the body would be so large that the distance across a cube of the same size would be approximately $(128{,}350)^{1100}$ light years. In cubic miles this would be about 10 with 840 zeros to the right of it—a body as large as the whole siderial universe. If the body grew only at the rate of growth during the fifth month before birth, when it merely doubles its size, and this rate continued until growth ceased in the adult, the size of the body would be about 10 to the 75th power times the size of the sun. Even if the rate of growth continued the same as that of the month immediately preceding birth, the size of the body in the adult would be 1,000,000,000 times that of the sun.

What actually takes place is that the ovum doubles its size 14 times in the first month of prenatal life—an increase of 1400 per cent. In the second month the increase is 1000 per cent, in the third 350 per cent, fourth 225, fifth 175, sixth 82, seventh 67, eighth 60, ninth 50, and tenth 33 per cent. For instance, if the fetus weighs 4 pounds at the beginning of the ninth month, it would weigh 6 pounds at the beginning of the tenth month and 8 pounds at birth.

After birth the growth in size is 300 per cent the first year, 25 the second, 11 the third, and 9 the fourth year. Growth continues after this at about the same rate until about the age of 11 years in girls and 12 years in boys, at which time it is accelerated up to 16 in girls and 18 in boys. It decreases gradually after this until about the age of 23 years, when it has practically ceased.

The growth in length, or stature, is less than that in volume or size or weight. Before birth the length increases 900 per cent the first month, 350 the second, 120 the third, 100 the fourth, 30 the fifth, 25 the sixth, 20 the seventh, 12 the eighth, 10 the ninth, and 8 per cent the last month before birth.

After birth the increase in length is 100 per cent in the first five years, 50 per cent in the next ten years, and after that growth continues to maturity at a rate and to an extent that depends upon race, type, sex, habit, and conditions of life.

The first month of pregnancy, immediately after conception, is the most dangerous period because of the rapidity of growth in the ovum and the rapid transformations in the organs and parts of the embryo at this time. Influences extraneous to the ovum or embryo either from within or without the mother have a powerful effect during that period and many abnormalities and monsters may result from pathological conditions that supervene, as found by Stockard.

The first year after birth is likewise dangerous, and the highest morbidity and mortality rates are found during this period, except during the ages at which the oldest people die, when the death rate approaches and finally reaches 100. The first month of pregnancy and the first year of life after birth are the most important periods, and too great care cannot be given both the mother and guest during those periods.

The body somites, the heart, and the spinal cord grow most rapidly during the first month of prenatal life and the head and brain grow most rapidly during the second month. At this time also the extremities begin to bud from the body and their growth is rapid during the second month. During the third month the liver and thymus grow rapidly, and this is followed during the fourth month by a rapid growth of the kidneys and lungs. The fifth month sees a rapid growth of the spinal cord and brain stem, which is followed in the sixth month by a rapid growth of the cerebrum and torso. The latter continues to grow rapidly during the seventh month in connection with a rapid growth of the intestine and spleen. The ninth and tenth months see a rapid growth of the cerebellum, which continues over into the first year after birth. It may be significant that the rapid growth of the cerebellum precedes the rapid growth of the muscles and extremities, and that this takes place during the last months of pregnancy when the fetus is very active in its movements.

The lungs grow rapidly during the first year after birth, the teeth during the second, the heart during the third, and the kidneys during the fourth. The lungs begin to function immediately after birth and their growth is then rapid. The

teeth are coming through the gums during the latter part of the first year and throughout the second year, the last to appear being the second molars, which are large and make the second summer a danger period. The first year is more dangerous because during the first half the infant is becoming accustomed to a strange and unnatural environment in a cold, hard world, and in the second half the irruption of the teeth, combined with a linking up of the infantile and juvenile periods of growth, especially in premature infants, may cause metabolic insufficiency.

During the whole period of the first four years after birth the brain is growing rapidly, with a constant diminution of growth increment, and at the same time the head is also increasing in size. The shoulders also grow rapidly during the first four years, and the liver not only grows rapidly during that period, but its rapid growth continues throughout the next two years. The liver is active in the metabolism of digestion in this active growth period.

The stature increases rapidly from 4 to 8 years, first the torso at 5 and 6, then the head and pelvis at 7 and 8 years. The teeth are developing rapidly during the last two years and with them the face. The heart grows rapidly in connection with the activities of this period of rapid growth.

The alimentary organs and spleen, but especially the stomach, intestine, and liver, have a rapid growth period during the ninth and tenth years and the liver continues this its final period of rapid growth until the age of 12 to 14 years. The head and teeth grow rapidly during the eleventh and twelfth years.

The stature takes on its last period of rapid growth from 12 to 16 years, and at this time all the linear dimensions of the body increase rapidly. There is also a rapid growth of the heart, kidneys, lungs, brain, and muscles. Muscle growth is greater after puberty than before, and bone growth is greater before puberty than after. The heart has a final spurt in growth from 16 to 20 years, and this is the last period of rapid growth for any part of the body.

CONCLUSIONS

There can be no doubt about wave-like growth, and attention has been directed to this by numerous observers.

The approximate time periods of wave growth have been determined for some important structures, organs, and parts of the body.

I am at present working on differences as the result of race, sex, type, environment, and other factors, and there yet remains undiscovered the cause of the shifting of the locus of rapid growth from one part of the body to another.

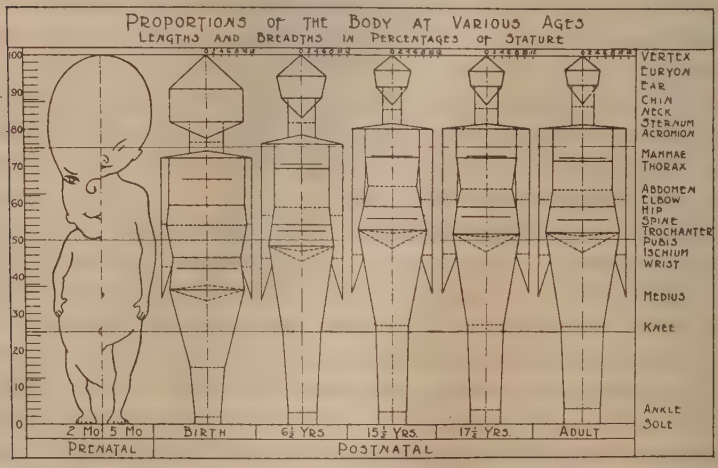

Chart 6 Proportions of the body at various ages. Modified from Godin. Attention is called to the greater length of the legs at 15½ years and the torso at 17½ years, in relation to stature.

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EXCEPTIONS TO BATESON'S RULES OF MINOR SYMMETRY

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FIFTEEN FIGURES

Basing his conclusions on an analysis of about 120 cases of reduplication in insect appendages and a number in the Crustacea, Bateson ('94) points out that there are two types of extra limbs: 1) Those in which an extra pair, symmetrical within itself, grows out from a normal limb, and, 2) those in which a single extra limb stands in mirrored relations with the normal. Among the cases considered by Bateson, limbs belonging to the latter category seem to be considerably in the minority since he stresses the former type as being almost universal.

The rules designed by Bateson (p. 479) to govern these abnormalities are as follows:

I. The long axes of the normal appendage and of the two extra appendages are in one plane: of the two extra appendages one is therefore nearer to the normal appendage and the other is remoter from it.

II. The nearer of the two extra appendages is in structure and position formed as the mirror image of the normal appendage in a plane mirror placed between the normal appendage and the nearer one, at right angles to the plane of the three axes; and the remoter appendage is the image of the nearer in a plane mirror similarly placed between the two extra appendages.

Transverse sections of the three appendages taken at homologous points are thus images of each other in parallel mirrors.

Among the vertebrates, also, Bateson cites a large number of cases, particularly in the group of amphibians. He lays very little stress upon the group of 'double' limbs in which

only two members stand in symmetrical relations, and implies that cases in which two limbs of the same asymmetry stand in series are practically non-existent.

When we consider the evidence afforded by transplantation experiments on amphibian larvae where we are supplied with a much more plastic and readily controlled material than that offered by the crustacea, conditions for interpretation become somewhat more propitious. Harrison ('18, '21) has shown how easily large numbers of reduplicated limbs may be produced in Amblystoma merely by reversing the anteroposterior axis of the embryonic limb at a very early stage of development. Under such experimental conditions, the first or primary limb which grows out from the transplanted disc is, in a large percentage of the cases, a limb of the other side of the body. This simple condition remains uncomplicated in only a small number of the cases because a secondary appendage usually grows out from some region of the primary limb and may acquire a degree of differentiation equal to or even considerably surpassing that of the primary.

So far as our observations go, secondary limbs formed by budding are invariably mirror images of the primary if the subsequent development of neither limb becomes complicated by rotation. It is known that the anteroposterior axis of the rudiment is determined at the time of operation (Harrison). It is also known that this anteroposterior axis coincides with the radio-ulnar axis of the definitive limb (Harrison, '18; Swett, '23). When the reduplicant is formed its anteroposterior axis (i.e., its future radio-ulnar axis) assumes symmetrical relations with the corresponding axis of the host (Swett, '24). This differentiation is irreversible. Furthermore, the dorsoventral axis of the limb is determined by its relationship to the tissue surrounding it (Nicholas, '22). The asymmetry of the definitive limb is not determined by either of these factors alone, but by a combination of the two.

It will be convenient to consider briefly at this time the phenomenon of rotation, first observed by Harrison in the development of a number of disharmonic limbs and later

worked out more in detail by Nicholas ('24). It was noticed that during the period of early development of limbs experimentally rotated rather less than 180° over their own dorsal semicircumference, certain changes in position with reference to the body wall were effected. Such limbs, instead of maintaining the posture imposed upon them at the time of operation, gradually recovered their normal (harmonic) posture. Various grades of partial recovery of posture by this method have also been observed, and an understanding of these, as well as a knowledge of the possibility of total recovery, is essential for an interpretation such as is here presented to account for several cases of atypical symmetry relationships. A certain amount of rotation may take place after the dorsoventral axis of the limb has become fixed, in such case an inverted limb is the obvious result.

When three or more limbs are formed, the definitive picture may become so complicated by reason of extensive fusion of the members or by resorption of one or more parts that the only hope of a rational interpretation lies in careful observations on the developmental stages.

In an excellent discussion of the question of minor symmetry as applied to reduplications occurring in transplanted limbs of Amblystoma, Harrison ('21) points out the necessity for a modification of the rules as set forth by Bateson. The relationship between double and triple limbs is particularly emphasized, inasmuch as the more recent work (since 1894) has shed much light on the origin of supernumerary appendages and has shown that reduplicated complexes with two members may be even more common under certain conditions than the conventional paired reduplicant of the original member so emphatically sponsored by Bateson. That these double limbs are not necessarily the result of suppression of an originally present third member has been established beyond question by careful observations on the developing limbs from the time of their first appearance.

Quoting Harrison ('21, p. 97)—

Bateson's rules might be stated in more general form, so as to include both simple duplicities and symmetrical pairs, as follows:

1. The long axes of duplex or multiplex appendages lie in one plane.

2. Two adjacent members form in structure and position the image of each other, as reflected in a plane mirror bisecting the angle between the respective axes and perpendicular to the common plane of the two axes.

As stated above, in practically every instance in which two or more limbs are produced where only one should have formed, the members of the complex obey the rules of minor symmetry. While the fundamental nature of this arrangement cannot be disputed, several exceptional cases have appeared in the literature[1] and others are available from the writer's collection of double-limbed Amblystoma larvae. Because these are somewhat different in appearance and in manner of formation, it seems advisable to defer extensive comment on them until each has been described briefly.

Case 1. A beautiful case has recently been reported by Dawson ('20) in which the components of a triple lobster chela (fig. 1) apparently furnish a clear-cut exception to the rules as originally propounded by Bateson. A symmetrical pair of chelae is here seen overshadowing the smaller normal chela on which it is borne. The normal appendage is of the smaller or 'nipper' type, while both members of the extra pair are of the larger or 'crusher' type. Furthermore, the nearer of the two extra appendages seems to be of the same asymmetry as the normal rather than its mirror image. According to the interpretation placed upon the case by Dawson, the appendages are arranged R–R–L, instead of alternating in the R–L–R order which would obtain were the members in strict adherence to the rules. Furthermore, Dawson states that the pigmentation on the ventral surface of the extra pair is similar to that ordinarily seen on the dorsal surface, thus

[1] Many cases of extra limbs of various types have been described in the higher vertebrate classes, including man. These have been purposely omitted from the present paper, although some of them are undoubtedly exceptions to the rules of minor symmetry. There seems to be no reason for placing them in a separate category or for devising for them any explanations not equally applicable to other reduplications.

indicating that the supernumerary members may be upside down. The significance of this point will be discussed later.

Case 2, 3 (Rem. E. 17; H.R.E. 10; Harrison, '18). In each of these cases two limbs of the same asymmetry stand in series (figs. 2 and 3). They resulted from experiments involving removal of the anterior half of the limb rudiment, and in commenting on the cases Harrison says (p. 441):

The case of the single limb with spur (Rem. E. 17) is interesting in that the spur developed out of a bud which grew posterior to the wound scar and which at first seemed to be the main limb. Anterior

Fig. 1 Supernumerary lobster chelae. Case 1. Redrawn from Dawson's ('20) fig. 1, p. 78. × ½.

to the wound a second bud appeared, ... finally developed into a normal limb of the proper laterality, the posterior bud remaining attached to it as a spur. . . . . The case with two independent limbs (H. R. E. 10) is fundamentally similar . . . . The main limb developed posterior to the wound. Later there appeared anterior to this a second bud. In this case, however, the two buds remained permanently separate . . . . the posterior member obviously arose from the remaining half of the limb bud after operation, while the anterior one regenerated from the anterior border of the wound, the two remaining far enough apart not to influence one another.

Case 4 (H.R.E. 20; Harrison, '21). This specimen also shows two right limbs standing in series on the right side of the body (fig. 4). These were formed in a different manner from the first two, having resulted from an experiment in

which the dorsal half of a left rudiment was substituted for the dorsal half of a right. One of the limbs developed from the transplant and recovered its normal posture by rotation, the other probably grew out from the stationary ventral half of the original limb.

Case 5, 6 (Hom. d.v. 54; Hom. d.v. 46; Swett, '24). In each of these cases an inverted right limb disc transplanted in orthotopic location reduplicated on its posterior border. This extra member became an abnormal right limb. The primary limb rotated back to assume a normal posture and became a perfectly normal right (fig. 5).

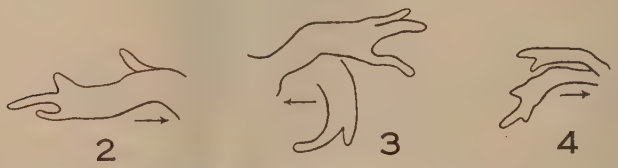

Fig. 2 Amblystoma. Experiment Rem. E. 17. Double right fore limb case 2. Redrawn from Harrison's ('18) fig. 33, p. 440. Lateral view. × 17. Arrow points anteriorly.

Fig. 3 Amblystoma. Experiment H.R.E. 10. Two left fore limbs. Case 3. Redrawn from Harrison's ('18) fig. 34, p. 440. Lateral view. × 17. Arrow points anteriorly.

Fig. 4 Amblystoma. Experiment H.R.E. 20. Two right fore limbs. Case 4. Redrawn from Harrison's ('21) fig. 132, p. 79. Lateral view. × 10.

Case 7 (Hom. d.v. 15; Swett, '24). This abnormality is the result of the same type of operation as the preceding, but the behavior of the limbs during development and their relations to each other differ somewhat. The primary limb (disharmonic) rotated part way back to normal posture and then reduplicated on its ulnar border. Both limbs are rights (harmonic) in their definitive condition, the primary is upside down and the secondary right side up. The former is held in this abnormal position by reason of the close fusion with its mate (fig. 6).

Case 8 ($AA2S_{73}$; Detwiler, '22). The two practically normal harmonic limbs which are exhibited in this case developed from a single rudiment transplanted to the gill region.

Serial sections showed that they were separated by a cartilaginous structure interpreted by Detwiler as an aberrant branchial bar, and it seems probable from the arrangement of the girdle tissue that the original rudiment was split into two by the interposition of this barrier, each half developing independently of the other into a normal limb[2] (fig. 7).

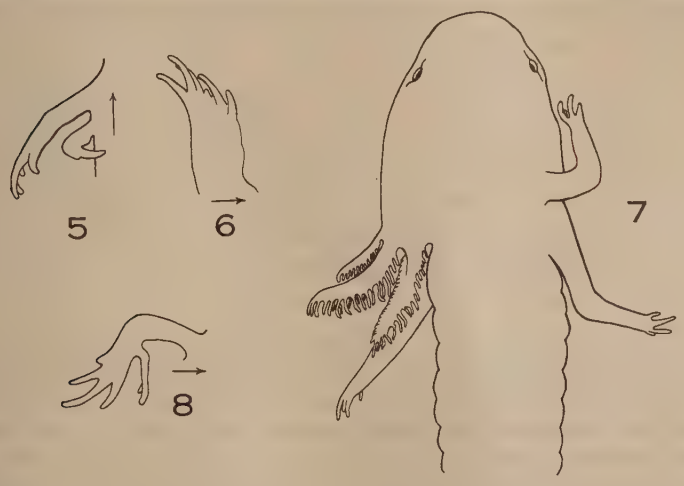

Fig. 5 Amblystoma. Experiment Hom. d.v. 46. Two right fore limbs. Case 6. Ventral view. × 11.

Fig. 6 Amblystoma. Experiment Hom.d.v. 15. Two right fore limbs. Case 7. Palm of anterior member partly faces dorsum of posterior. Ventral view. × 11.

Fig. 7 Amblystoma. Experiment $AA2S_{73}$. Two right fore limbs in gill region. Case 8. Redrawn from Detwiler's ('22) fig. 17, p. 138. × 5.

Fig. 8 Amblystoma. Experiment A38 r/u 21. Two right fore limbs. Case 9. Lateral view. × 11.

[2] In connection with this case may be mentioned the experiments of the present writer ('24) in which the embryonic rudiment was split artificially in various planes. Although no examples so simple as this case of Detwiler's were obtained, the results show conclusively that each primary limb developing from either half of a rudiment so divided is harmonic, although in practically every case one or the other produced a symmetrical reduplicant. Halves of limb rudiments behave in such cases exactly like separate and distinct whole rudiments placed rather close together.

Case 9 (A38 r/u 21; Swett, '24). At the time of operation the limb bud was in stage 38 (Harrison, '18), and after it was split wide open with the scissors a fragment of indifferent tissue was healed in place to prevent union of the halves. One limb grew out on each side of the interposed barrier, and the developmental stages show that both are rights (harmonic). Even though one member subsequently became considerably reduced in size, it retained definite characteristics of its asymmetry (fig. 8).

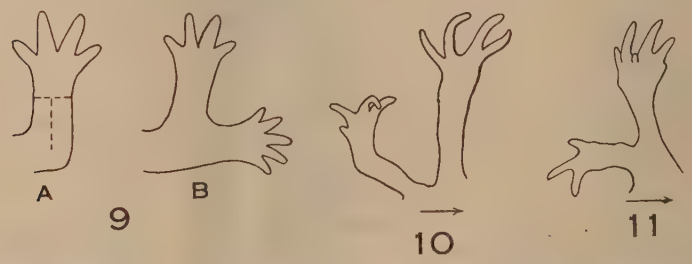

Fig. 9 Triton alpestris. Case 10. A. Normal right fore limb, showing direction of cuts resulting in—B. double limb. Dorsal view. Redrawn and reduced one-half from Fritsch's ('11) fig. T', p. 465.

Fig. 10 Amblystoma. Experiment Hom. d.v. 146. Case 11. Triple limb. Posterior member normal right, inverted anterior complex of two limbs mirrored in ulnar plane. Ventral view. × 11.

Fig. 11 Amblystoma. Experiment Hom. d.v. 9. Case 12. Triple limb. Posterior member normal right, inverted anterior complex mirrored in radial plane. Lateral view. × 11.

Case 10 (Fritsch, '11). This reduplication resulted from the 'T–Schnitt' operation of Barfurth (fig. 9, A), and though no mention of the asymmetry of the components (fig. 9, B) is made in the text, the figures seem to indicate that they are alike. The anterior member is seemingly identical with the normal, and a figure given by the author (fig. U') but not reproduced here definitely shows the posterior member to be a right. The case is mentioned here because of its apparent similarity to the preceding (no. 9).

Case 11 (Hom. d.v. 146; Swett, '24). Three limbs are present, the most posterior is a harmonic member and is perfectly

normal. It is entirely separate from the anterior complex which is composed of two limbs closely fused along their ulnar borders. This complex is upside down. As they stand, the relationship between the three limbs is $\frac{R-R-L}{N \quad \text{Inverted}}$ (fig. 10).

Case 12 (Hom. d.v. 9; Swett, '24). This limb complex (fig. 11) also is composed of three members, of which the two anterior are inverted. The differences between this case and the one just cited hang on the mirror relationship between the members of the anterior complex. In case 11 the limbs are mirrored in the ulnar plane; in this one they stand in radial relation to each other. As before, inversion was brought about by an incompleted attempt to recover normal posture by rotation. The symmetry relationships here may be expressed as follows $\frac{R-L-R}{N \quad \text{Inverted}}$, and it will be seen that the case is the exact antithesis of the one just preceding, inasmuch as, if the inverted condition of the anterior complex could be corrected, the rules of symmetry would be violated, i.e., $\frac{R}{N}\frac{R-L}{N \quad N}$ (see p. 75).

Case 13 (38 ML Het. d. d. 4). This case has never been described and appeared in the course of experiments designed for another purpose. The left limb bud of one animal (stage 38) was transplanted to the right flank of another of the same stage. The undifferentiated mediolateral axis of the bud was reversed at the time of operation so that the combination is harmonic. During development it was noted that the anterior and middle members of the triple complex were both lefts. In its definitive condition forty-two days after operation the middle limb may be seen to have undergone considerable reduction, but the two outside members are definitely mirror images of each other. The scheme is thus $\frac{R-L-L}{N \quad \text{Irreg. N}}$. The developmental history of this case shows beyond a reasonable doubt that the middle limb is a left, but, whatever its asymmetry, the mere presence of a third member between the components of a mirror-imaged pair conclusively proves this to be an exception to the rules (fig. 12).

Case 14 (S.d.d. 13). The specimen (fig. 13)[3] resulted from a double operation involving artificial splitting of a limb rudiment later transplanted to another animal in disharmonic orientation (i.e., with the anteroposterior axis reversed). Two limbs of the same asymmetry are present, each nearly normal except for some hyperdactyly, the anterior one is

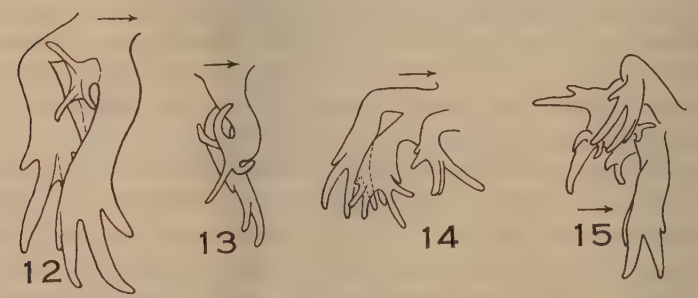

Fig. 12 Amblystoma. Experiment 38ML Het. d. d. 4. Case 13. Triple limb in heterotopic location. Small limb between members of normal imaged pair. Lateral view. × 14.

Fig. 13 Amblystoma. Experiment S. d. d. 13. Case 14. Two right limbs, the more posterior and dorsal inverted, the other normal. Dorsal view. × 9.

Fig. 14 Amblystoma. Experiment S. d. d. 11. Case 15. Quadruple limb. The anterior and posterior members are normal rights, the middle complex a mirrored pair. Anterolateral view. × 9.

Fig. 15 Amblystoma. Experiment S. d. d. 17. Case 16. Quadruple limb. Anterior right normal, ventral left normal, posterior complex abnormal and mirrored in radial plane. Anterolateral view. × 9.

right side up, the posterior inverted $\frac{R}{\text{Inverted}} - \frac{R}{N}$. This case is obviously similar to nos. 4 and 8, inasmuch as each limb arose independently from a half disc.

Case 15 (S.d.d. 11). In this case we have a quadruple limb produced after an operation exactly similar to the one which caused the double appendage in no. 14 to form. The complex (fig. 14) may be expressed by the formula $\frac{R}{N} - \frac{R}{N} - \frac{L}{N} - \frac{R}{N}$. The more dorsal right limb arose from the portion of the trans-

[3] No explanation of the apparent reversal of asymmetry shown by certain of the primary limbs in cases 14, 15, and 16 can be given at this time. We are here considering the symmetry relations of the members without reference to their derivation.

plant anterior to the barrier, the closely fused double complex from the other half of the limb disc posterior to the barrier, and the most ventral limb is probably a regenerate from the tissues of the host.

Case 16 (S.d.d. 17). This specimen (fig. 15) again shows a quadruple limb resulting from the same type of operation as the preceding. The components are arranged somewhat differently, however, and may be expressed by the formula: $\frac{R}{N}-\frac{L}{Abn.}-\frac{L}{N}-\frac{R}{N}$. The definitive condition of the limb is very complicated, but the appendage which developed anterior to the barrier is definitely a right, the complex produced from the transplant posterior to the barrier is symmetrical within itself, consisting of an anterior left and a posterior right component. That the fourth limb, situated near the base of the complex, is a left (disharmonic) indicates that it arose from a portion of the transplanted bud rather than from the tissues of the host.

DISCUSSION

If we consider the foregoing group of abnormal limbs without reference to their derivation or the peculiarities of their development, formulation of a plausible interpretation of their relationships becomes virtually impossible. Fortunately, however, we are provided with developmental data ample for our purpose and we are enabled to draw certain very interesting analogies and conclusions.

For convenience we may first discuss the exceptions to the rule which are composed of only two limbs. These are not only the simplest in their definitive condition, but inasmuch as the steps in their development are uncomplicated by the presence of a third or fourth member they are somewhat more easily followed. On the basis of their derivation, we are immediately tempted to segregate the cases into two groups: 1) those resulting from direct experimental splitting of the limb bud or from other purely fortuitous initiation of two independent growth centers, and, 2) those derived from a single rudiment by a process of spontaneous budding. Most

unfortunately this distinction is open to criticism on account of our lack of knowledge of the ultimate causes of spontaneous reduplication. In spite of very good evidence that both primary limbs developing from isolated portions of a limb bud always exhibit the same asymmetry and that a secondary limb formed by budding is a mirror image of the primary, the boundary line between these two methods of double limb formation is very indistinct. Undoubtedly the true reduplicating bud is formed by a division of the original into two by some unknown factor, the action of this or of some other concomitant factor being at the same time responsible for the reversal of asymmetry in the secondary member.

However, since Harrison ('18) has shown that the primitive limb disc is an equipotential system, it becomes obvious that an extra limb developed from an isolated portion of the original rudiment can no more appropriately be considered a reduplicant when its anlage remains close to the normal location than when it is transplanted to some other more distant region of the body. In either case the symmetry relations between the limbs do not differ materially from those observed when an entire rudiment from another animal is transplanted in a corresponding heterotopic location. Cases nos. 2, 3, 4(?), 8, 9, 10, and 14 belong to the group in which the rudiment was artificially divided. In each case, with the possible exception of no. 10, the resultant limbs are not mirrored, but stand in series. The apparent contradiction between this statement and the published results of certain other investigators has been commented on elsewhere and need not concern us here.

On the other hand, we do meet, in cases where the rudiment has not been separated by operative procedure into two isolated growing regions, interesting combinations of reduplication and rotation phenomena. Cases no. 4(?), 5, 6, and 7 show how inextricably these two processes are involved and the part each may play before the limbs reach their final posture. Reduplication of the original bud preceded its complete recovery of normal posture in the first three instances, while in the fourth a partial rotation of the primary limb was ac-

complished before the secondary bud appeared. The close fusion of the two accounts for the inverted condition of the primary. It is admittedly possible that one of the two limbs in each of these cases was derived from the transplant and the other from the tissues of the host, but it seems that we are justified in minimizing the importance of regeneration here because of the careful cleaning to which the wound bed was subjected prior to transplantation (Harrison, '18).

Certain of the triple limbs (cases nos. 11 and 12) are wonderfully instructive when interpreted on the basis of their development. It was pointed out above that these two cases possess limb complexes of quite different relationships. The former is an exception to the rules as it stands $\overset{(R-R-L)}{N\ \text{Inverted}}$, but if the anterior complex could be restored to its normal posture by rotation as a whole, the rules would be diagrammatically exemplified. The latter is in accordance with the rules as it stands, $\overset{(R-L-R)}{N\ \text{Inverted}}$ being abnormal only in the inversion of the anterior complex, but could this be rotated as a whole to normal posture the rules would be violated. This particular reduplication (no. 12) is especially valuable in considering the relationships of the supernumerary pair of lobster chelae reported by Dawson and described in the present paper as case 1. Dawson's discussion makes considerable point of the inversion of the extra claws without noting that if they actually are inverted the case is not an exception to the rules of symmetry. If the increased pigmentation of the ventral surfaces of the paired reduplicant in this case does prove it to be inverted, the arrangement is $\overset{R-L-R}{\text{Inverted}\ N}$, identical with the sequence in my own case no. 12. If the contrary is true, as seems doubtful, and the extra claws are not inverted, the case is a true exception to the rules with the arrangement $\overset{L-R-R}{N\ N\ N}$, in this event the exact counterpart of my own case no. 11. Reversal of the dorsoventral axis in this case and the relationship which this reversal bears to the asymmetry of the various components are commented on by Dawson as follows: "The extra bud, due to mechanical dis-

placement of its tissue at the time of first injury, or as a result of a subsequent accident, has had its dorsoventral orientation reversed, causing the development of twin crushing claws which are mirror images of each other'' (p. 81). It is indeed quite possible that the inversion was brought about by injury with consequent displacement of tissue, but, judging from work done on amphibian material, it seems doubtful if great stress can be laid on reversal of the dorsoventral axis as a cause of symmetrical reduplication. It is, however, entirely possible that conditions are different in the crustacea, inasmuch as Przibram ('10) has found that dorsoventral differentiation is especially well marked in animal organization.

At all events, we have positive evidence of the mechanism by which single limbs and limb complexes may be inverted in Amblystoma, and thus we are furnished a means of interpreting these cases whose causative factors must otherwise have remained obscure.

The one case (no. 13) in the present series which developed in heterotopic location is of some interest for that reason (fig. 12). There is no question here that all the limbs were produced from the transplant. Regeneration from the host was impossible. The uncertainty comes when we try to explain the occurrence of the middle member. Its asymmetry indicates that it arose by budding from the primary limb (the posterior), but in no previously reported case in the amphibia have two radial reduplicants been formed by the same limb. The asymmetry, the position, and the existence of the middle member of this complex are most anomalous. The marked reduction which it underwent probably took place as a result of faulty nutrition rather than from any incompatibility with its neighbors. The blood supply to even a single limb in such an unusual region may very reasonably be expected to be inadequate, and a glance at other limb complexes in this group will show that two limbs of the same asymmetry may exist together in perfect mutual harmony.

From these cases we are permitted to conclude that two limbs of the same asymmetry may stand in series either, 1)

as a result of independent origin and development from isolated fragments of the same or different harmonic limb rudiments or, 2) in case one or more arises from a disharmonic rudiment, in consequence of successive rotation and reduplication phenomena.

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THE ABSENCE OF SUPPLEMENTARY PRISMS IN HUMAN ENAMEL

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TWO FIGURES

It is well known that, while many of the enamel prisms or rods of the human dental enamel are approximately straight, the majority have a spiral or undulating course. Furthermore, they are arranged in small bundles, the members of which are roughly parallel. These bundles cross one another at various angles producing an irregularity of arrangement which makes it exceedingly difficult to trace individual prisms through their full extent.

This difficulty is responsible for the controversy regarding the structural basis for the increase of area at the external surface of the enamel over that of the dento-enamel junction.

Although, as far as available literature shows, no one has ever demonstrated with certainty that most of the prisms extend from dento-enamel junction to the external surface, this condition has been tacitly and almost universally accepted as correct by writers on the histology of teeth.

If we admit, a priori, that most of the enamel prisms do extend from dento-enamel junction to surface, there are three possible explanations[1] of the fact that the area of the external surface is greater than that of the surface adjacent to the dentin. These are:

1. All prisms extend through the entire thickness of the enamel. Increase of surface results from the increase in diameter of individual prisms from proximal to distal.

[1] For a fourth possible factor see the explanation given by Noyes, referred to below on p. 81.

2. Most prisms extend through the entire thickness without increase of diameter. Increase of surface is accomplished by the addition of supplementary prisms distally.

3. Most prisms extend through the entire thickness, increasing in diameter, but not sufficiently to account entirely for the greater area at the surface. The difference is provided by supplementary prisms added distally.

There are advocates of each of these explanations, some adopting them wholly, others only partially. Only a few illustrative views are given here.

In favor of the first explanation were von Ebner ('99) and Pickerill ('13). Von Ebner expressed the opinion that the enamel prisms extend from dento-enamel junction to external surface and found a very considerable increase in diameter of individual prisms from proximal to distal. He did not mention the subject of supplementary prisms.

Pickerill, by measurement of many prisms from teeth of New Zealanders and Europeans, found an average proximal diameter of 3.1 μ and an average distal diameter of 5.7 μ for prisms over the buccal curve. The ratio of these averages, 1 : 1.83, when compared with the ratio of inner to outer surface of the enamel, 1 : 1.76, gave very strong evidence against the possibility of the presence of supplementary prisms.

Mummery ('16) found dichotomously branching prisms with supplementary prisms introduced between them in the enamel of the compound third molar of the wart-hog (Phacochoerus) and ('19) thought it probable that a similar condition is found in human enamel, though he gave no evidence for this inference.

Andrews ('19) contended that Pickerill ('13) was in error and claimed to have seen many supplementary prisms in developing enamel. He counted nineteen prisms in a given extent at the dento-enamel junction and twenty-four in an equal extent nearer the surface and believed that this meager observation proved the existence of supplementary prisms. He conjectured that the additional prisms are formed by new ameloblasts derived from the cells of the stratum intermedium of the enamel organ.

Noyes ('21) stated very emphatically that the enamel prisms "are of the *same diameter at their outer and inner ends*" (p. 41). Besides claiming the presence of many supplementary prisms, especially over the more convex parts of the crown, he ascribed the increase of area to the fact that, though the prisms are at right angles to the dento-enamel junction, they are seldom at right angles to the outer surface. He illustrated this by the analogy of a stack of paper cut obliquely, the obliquely cut surface having a greater area than one cut perpendicularly.

Dewey ('14), Tomes ('14), Broomell and Fischelis ('22), and others have favored the view of supplementary prisms.

In Lewis and Stöhr ('14) the third explanation was favored, admitting a slight enlargement of the prisms distally, besides a distal thinning of the interprismatic substance. Supplementary prisms were regarded as possible.

By far the majority of writers on the subject have leaned toward the view that the increase of surface area is accomplished by the addition distally of supplementary prisms, with or without increase in diameter of the other prisms which extend the entire distance from the dento-enamel junction to the surface of the crown. Very few investigators have cited actual evidence in favor of their contentions.

It is the purpose of this paper to report briefly tests which, it is believed, prove that the increase in area at the surface of the crown over that at the dento-enamel junction is due almost entirely to increase in diameter of the enamel prisms and that supplementary prisms do not occur in human dental enamel.

All of the sections used in these investigations are from human teeth of the permanent dentition. They include labiolingual and transverse sections of upper incisors and cuspids and a mesiodistal section through the buccal cusps of an upper first molar.

The sections were ground to approximately 20 μ on a grinding apparatus similar to that described by Noyes ('21). Two methods were employed:

1. A modification and extension of the method of Pickerill ('13). Measurements were made of the outer and inner ends of a great many prisms and the average inner and outer diameters computed for each section. The ratio of the average inner diameter to average outer diameter was then compared with the ratio of length of the dento-enamel junction to length of the outer surface of the enamel in the same section.

2. A method in which the number of prisms in a section, or in a part of a section, was counted, first at the outer surface of the enamel, then at the dento-enamel junction.

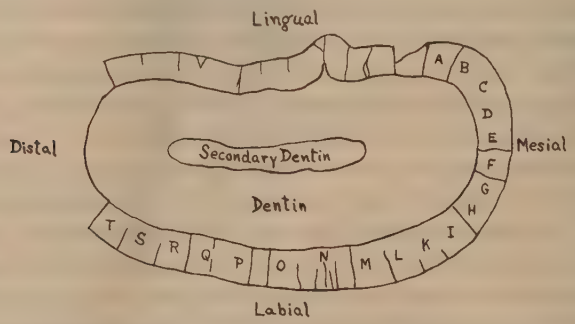

Fig. 1 Transverse section of permanent upper central incisor, through occlusal third. Drawing made with aid of projection apparatus. × 8.67.

The measurements were made with the aid of a calibrated ocular micrometer, the sections being viewed with a 10x ocular and a 4-mm. objective. In making the measurements, the number of prisms covered by twenty divisions of the micrometer scale was counted and the average diameter of the prisms in such a group computed. Obviously, in such a method the measurement includes the thickness of the interprismatic substance. Furthermore, the greatest width of every prism does not enter into the measurement because often a prism is cut to one side of its longitudinal axis. For these reasons the figures given in the record cannot be taken to represent the actual width of the enamel prisms. For the purposes of this paper, however, they are pertinent, since the measurements

are to be used in computing only the ratio of inner to outer diameters of the prisms, and the same variables affect both measurements in a similar manner.

The results obtained by these methods are strikingly uniform. Two typical analyses will serve adequately as exam-

TABLE 1

Analysis of the section shown in figure 1

SEGMENT	AVERAGE MEASUREMENT, PRISMS		RATIO OF PROXIMAL TO DISTAL MEASUREMENTS	PERCENTAGE OF INCREASE DISTALLY
	PROXIMALLY	DISTALLY		
	μ	μ		*Per cent*
A	5.09	6.47	1: 1.27	27.
B	4.85	6.88	1: 1.44	44.
C	4.72	6.97	1: 1.52	52.
D	4.85	6.22	1: 1.28	28.
E	4.85	6.73	1: 1.38	38.
F	5.09	5.98	1: 1.17	17.
G	5.09	6.41	1: 1.26	26.
H	5.41	5.91	1: 1.09	9.
I	4.72	6.66	1: 1.41	41.
A–I	4.93	6.47	1: 1.314	31.4
K	5.47	6.61	1: 1.18	18.
L	5.63	5.93	1: 1.06	6.
M	5.08	6.26	1: 1.23	23.
N	4.95	5.95	1: 1.20	20.
O	5.14	6.25	1: 1.21	21.
P	4.90	6.01	1: 1.23	23.
Q	5.25	5.14	1: 0.98	— 2.
R	5.26	6.21	1: 1.18	18.
S	5.36	6.11	1: 1.12	12.
T	5.07	6.45	1: 1.27	27.
K–T	5.21	6.09	1: 1.168	16.8
A–T	5.08	6.28	1: 1.236	23.6

ples. The first of these concerns a portion of the enamel in a transverse section through the occlusal third of the crown of a permanent upper central incisor. In this section (fig. 1) the enamel was 'checked' and cracked in numerous places, these artefacts causing the loss of the enamel from the distal surface and the division of the remaining enamel into numerous segments. The enamel on the lingual aspect was broken

in some places and gnarled in others, so that a continuous area for analysis was not obtainable. The enamel over the mesiolingual angle, mesial aspect, mesiolabial angle, and labial aspect, however, was of regular arrangement and intact except for the numerous checks indicated in the diagram (fig. 1). This area, comprising approximately one-half the periphery of the section and including in it two markedly convex regions (the line angles), as well as a very flat region (the labial surface), was selected for analysis.

The region described above was divided, with the aid of the checks (which parallel the course of the prisms), into nineteen segments, lettered from A to I and from K to T, each segment subtending an arc of about one-half millimeter at the periphery. It will be noted that segments A to I include the more convex parts of the section and segments K to T the flatter parts. In each of these segments there were taken four measurements of the prisms at the dento-enamel junction and four at the periphery. The average of the outer measurements was then compared with the average of the inner measurements, as shown in table 1. An examination of this table shows that, in all segments except Q, there is an increase in diameter of the prisms from proximal to distal, the difference ranging from — 2 per cent in Q to + 52 per cent in C, with an average increase of 23.6 per cent, which shows a consistently greater diameter distally for the prisms.

If the increase in surface is to be accounted for wholly by this increase in diameter from proximal to distal, there would be expected not only a consistent increase, but also a greater percentage of increase over the more convex portions of the tooth. Table 1 demonstrates that this is the case, the average increase in segments A to I being 31.4 per cent—almost double the 16.8 per cent increase in segments K to T.

Furthermore, if the increase in surface is due to the increase in diameter of the prisms only, the ratio of average inner diameters of prisms to average outer diameters should be equal to the ratio of inner to outer surfaces of the enamel. Since the ratio of surfaces can be expressed by the ratio of

the length of inner border to outer border of the enamel, the following equation should be found true:

Average inner diameter of prisms: Average outer diameter of prisms = Length of inner border : Length of outer border.

The values of the lengths at the outer surface and at the dento-enamel junction were obtained by tracing the image of the section projected by means of a microprojection lantern, then measuring the tracings with calipers, or by threads pinned along the tracings. In this method the great magnification of the image reduces the error of the measurement.

Such measurements showed the ratio of inner to outer surfaces to be 1: 1.270, while the ratio of inner to outer diameters of prisms is 1: 1.236. This proves the equation given above to hold true within reasonable limits of error in microscopical measurements. Stated in percentages, the outer surface is 27 per cent greater than the inner, while the increase in average diameter of the prisms distally is 23.6 per cent—a discrepancy of only 3.4 per cent.

Finally, if each prism extends through the entire thickness of the enamel and increase of surface results from increase in diameter of the prisms alone, the number of prisms would of necessity be the same at the dento-enamel junction and at the surface. Actual counts made with the aid of a pointer in the ocular and a mechanical stage gave, for the enamel in segments A to T, 1690 prisms at the outer surface and 1693 at the dento-enamel junction. If all the prisms were of equal diameter throughout, it would require 469 supplementary prisms of a size equal to the average proximal diameters of the prisms in this section to account for the 27 per cent increase of periphery over inner border.

That similar results are obtained in longitudinal as well as transverse sections is demonstrated by the tabular analysis of a mesiodistal section through the mesiobuccal cusp of an upper first molar (fig. 2 and table 2, *b*). In this section the mesiobuccal cusp is sharply separated from the distobuccal by a very deep buccal groove. The area selected for analysis

in this case was divided into small segments by India-ink dots placed on the cover-glass in the positions indicated in figure 2. The average increase in diameter of the prisms in this cusp is 34.6 per cent, while the increase of surface was computed as 36.3 per cent—an error of only 1.7 per cent. The number of prisms counted at the dento-enamel junction is 1376; counted at the periphery, 1380.

The fact that, in both of the examples given above, the increase of surface is computed as slightly greater than the

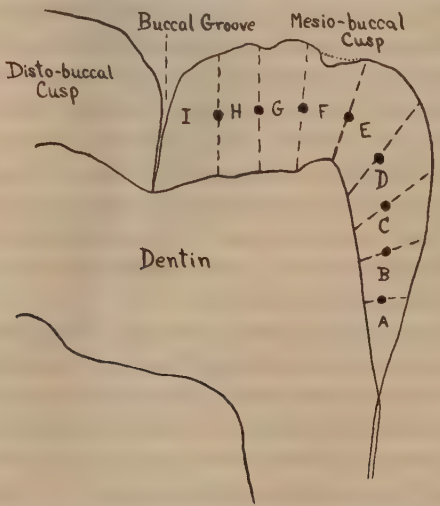

Fig. 2 Mesiodistal section through buccal cusps of upper first molar. Shows method of division of enamel into segments by placing India-ink dots on the cover-glass. Drawing made with aid of projection apparatus. × 8.67.

average increase in diameter of the prisms is not significant, for the converse is found with equal frequency. This is illustrated by the results of five typical analyses, as given in table 2. These results show a close agreement between increase in diameter of prisms and increase in surface; also a practically absolute correspondence between the numbers of prisms proximally and distally.

As suggested by Noyes ('21) (p. 81), the fact that some prisms meet the surface of the crown at acute angles, whereas

they are more frequently perpendicular to the dento-enamel junction, probably is a factor in the increase of surface. That it is an important factor, however, seems doubtful, since by far the majority of prisms are very nearly or quite perpendicular to the outer surface and the inclination of those prisms which do meet the surface at acute angles is not sufficient to cause more than a very small percentage of the increased surface.

TABLE 2

Analyses of five typical sections

SECTION	AVERAGE MEASUREMENTS, PRISMS		PERCENTAGE INCREASE DISTALLY	PER CENT INCREASE, OUTER OVER INNER SURFACE	NUMBER OF PRISMS	
	PROXIMALLY	DISTALLY			PROXIMALLY	DISTALLY
a. Transverse, upper central incisor. Figure 1.	5.08	6.28	23.6	27.0	1693	1690
b. Mesio-distal. First molar. Figure 2.	4.76	6.41	34.6	36.3	1376	1380
c. Transverse. Upper cuspid. About $\frac{1}{5}$ of enamel analyzed	4.80	5.79	20.6	18.1	1130	1139
d. Labiolingual. Upper lateral incisor.	4.99	5.88	17.8	17.0	3542	3510
e. Labiolingual. Upper cuspid.	4.67	5.83	25.0	22.2	Impossible to count prisms in this section.	

To investigate the possibility of the interprismatic substance playing some part in the increase of surface, this substance was observed and measured proximally and distally in many sections. It was found to vary markedly in thickness from point to point in the same section, as well as in sections from different teeth. In places where varicosities in adjacent prisms are contiguous there is an amount too small to be measured by the methods used. In hypoplastic areas the thickness is sometimes as great as 2.4 μ. However, the variability was found to be as great proximally as distally and

the average thickness near the dento-enamel junction is equal to that near the surface. Hence the interprismatic substance is a negligible factor in the increase of surface.

Since there are no supplementary prisms added during the development of the human enamel, it is unnecessary to postulate the augmentation of the number of ameloblasts by cells from the stratum intermedium. For the same reason each prism in human teeth must be the product of a single ameloblast.

CONCLUSIONS

1. The results corroborate the findings of Pickerill ('13) and prove that there are no supplementary prisms in human dental enamel.

2. The increase in surface of the enamel is accomplished almost entirely by increase in diameter of the prisms.

3. A very minor factor in the increase of surface is due to the fact that some of the prisms meet the outer surface obliquely.

4. The interprismatic substance plays no part in the increase of surface.

5. All the prisms in human dental enamel extend through the entire thickness of the enamel.

6. Each prism is the product of a single ameloblast.

7. Ameloblasts are not augmented numerically by cells from the stratum intermedium.

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A NOTE ON THE PHAGOCYTIC ACTIVITY OF BONE CELLS

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ONE FIGURE

There is at present a growing belief based on experimental evidence that the bone cell is active in organizing the matrix of its own environment. The earlier evidence for this belief is given in my previous papers on the bone cell (Am. Jour. Anat., vol. 29, no. 2, 1921, and vol. 29, no. 3, 1921). The most recent work is that of James Crawford Watt, University of Toronto. In his abstract in The Anatomical Record, vol. 27, no. 4, 1924, he writes: "No evidence was found tending to the conclusion that the salts were deposited in the bone matrix by precipitation but support was found for the opinion that the salts in the matrix are deposited as a secretory phenomenon of the osteoblasts."

The experimental evidence, which has given occasion for this note, indicates that the activity of the bone cell is not limited to the building of its environment, but that under certain conditions it may be phagocytic in its nature. Two classes of phagocytes are commonly described: microphages, small polymorphonuclear leucocytes which ingest chiefly bacteria, and macrophages, largely scavengers ingesting dead tissues and degenerated cells. The latter are produced mainly by proliferation of fixed connective-tissue cells. Certain recent investigators go far beyond this limit and attribute phagocytic function to endothelium and even epithelium. However, to my knowledge a cell of mesodermal origin, which has developed into a special tissue cell has never been observed to

perform a phagocytic function. Areolar connective tissue is not considered a specialized tissue because of its diversified cellular content. The purpose of this paper is to show that a specialized tissue cell like the bone cell can perform a phagocytic function.

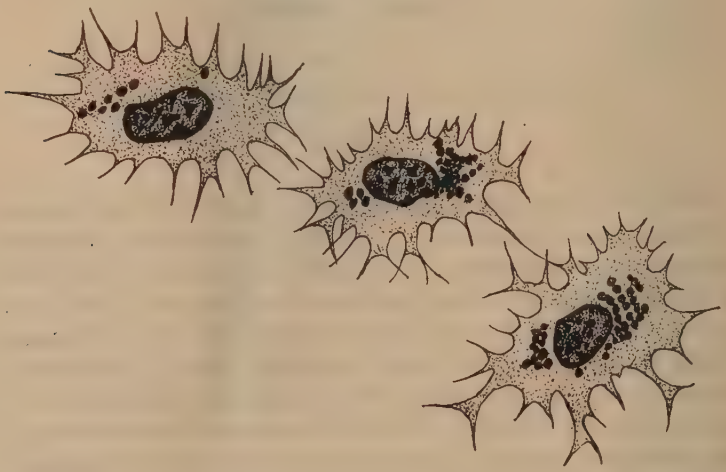

Fig. 1 Bone cells containing India-ink droplets. Camera-lucida drawing. × 1200.

MATERIAL AND TECHNIQUE

Young rats, one to seven days old, were employed for these experiments. Observations on a large series showed that the parietal bones of such animals were definitely ossified. The parietal bone was chosen because it was thin enough for study without subjecting it to the decalcification and section method. A small amount of India ink was placed in the bone by means of a fine hypodermic needle. The animals were sacrificed after three to ten days. The bone was then prepared according to the technique described in my previous papers on bone cells, and studied with an oil-immersion objective.

OBSERVATIONS

The preparations revealed the fact that the bone cells in the area where the India ink had lodged contained many droplets of the ink (fig. 1). In areas where little ink was present it was found in the cells of the haversian canals and in the bone cells, while none was found in the bone matrix. In areas where much ink was present the cytoplasm of the bone cells was packed with it, the matrix immediately surrounding them contained none, while the matrix more removed showed a diffuse granular deposit of the ink pigment.

In the preparations from rats killed three to five days after injection the protoplasm of the ink-containing cells appeared normal, while in animals killed seven to ten days after injection the cells containing large quantities of the ink showed marked signs of degeneration. In all of these preparations the site of injury showed a marked increase of vascular tissue.

I have cited these observations not with the intent of presenting evidence that any cell no matter how specialized may under certain conditions perform as a phagocyte, but simply to present the interesting fact that a specialized cell like the bone cell is active not only as a builder, but also as a protector even at the hazard of its own life.

THE LESSER TROCHANTER OF THE MAMMALIAN FEMUR

ALFRED SHERWOOD ROMER
Walker Museum, University of Chicago

THREE FIGURES

The homologies of the femoral trochanters have been discussed by Gregory in 1918 and the writer in 1922. In mammals three trochanters may be present: 1) the greater trochanter, serving as a point of insertion for a portion of the gluteal musculature; 2) the lesser trochanter for the iliopsoas, and, 3) the third trochanter for a portion of the gluteal mass.

As shown by Gregory, the third trochanter is a distinctly mammalian structure and is not present in reptiles. In 1922 I presented evidence tending to show that the greater trochanter is also an independent development in the mammal-like reptiles and mammals, and had no predecessor in primitive reptiles, although other forms, such as dinosaurs, have evolved analogous structures (von Huene, '11; Gregory). The changes in the type of locomotion which have brought about the development of the mammalian trochanter have been discussed by Gregory and the writer.

The lesser trochanter of mammals presents greater difficulties. On the ventral[1] surface of the femur in many reptiles are found two trochanteric structures (fig. 1, a), both of which have been suggested as complete or partial homologues of the lesser trochanter. The more proximal is the inner

[1] In this paper the terms used for orientation are those of the primitive (reptilian) position of the tetrapod limb, ventral being the equivalent of the human posterior, dorsal that of anterior, anterior that of internal, and posterior that of external.

trochanter, which serves as a point of insertion of a portion of the pubo-ischiofemoralis externus (obturator externus), the remainder of the muscle inserting in the fossa posterior to it, much as does its mammalian homologue. More distally, along a ridge partially homologous with the mammalian linea alba, is situated the 'fourth trochanter,' into or beside which the coccygeofemoral muscles insert. Von Huene has attempted to homologize these two trochanters; but, as has been pointed out by Gregory and as may be seen from the condition in Dimetrodon, they are independent, although often adjacent structures. It is highly improbable, from a consideration of the musculature, that the fourth trochanter has played any part in the development of the mammalian trochanteric system.

The characteristic difference in the development of the tail in typical mammals as compared with typical reptiles is an obvious but usually unstressed point. The reptilian tail is typically a stout structure especially in its basal portion, where, on either side, two large muscles have their origin. These, the coccygeofemoral or caudifemoral muscles, insert into or near the 'fourth trochanter' of the femur and play an important part in reptilian locomotion. In the change to the mammalian type, these muscles have become inoperative to a great extent and have become reduced to small slips, the exact homologies of which are often uncertain (pyriformis, etc.). The tail of mammals, in contrast with that of reptiles, is slim and has lost its primitive locomotor function, although it is somewhat stouter in certain archaic types. The insertion of the coccygeofemoral homologues is usually in the neighborhood of the greater trochanter. These muscles in mammals have no landmarks of their own, and it is highly improbable that the fourth trochanter will be found in any mammal or closely related reptilian type. I have never seen a certain indication of a fourth trochanter in the mammal-like reptiles (Therapsida of Broom), although the ventral ridge ('linea alba' in part) on which it was originally situated is of course present.

There remains, then, as a possible predecessor of the mammalian lesser trochanter, the reptilian internal trochanter. If a primitive reptile and a 'modern' type of mammal be compared directly (fig. 1, a and h), the two are not essentially dissimilar in position and appearance. In most mammals the

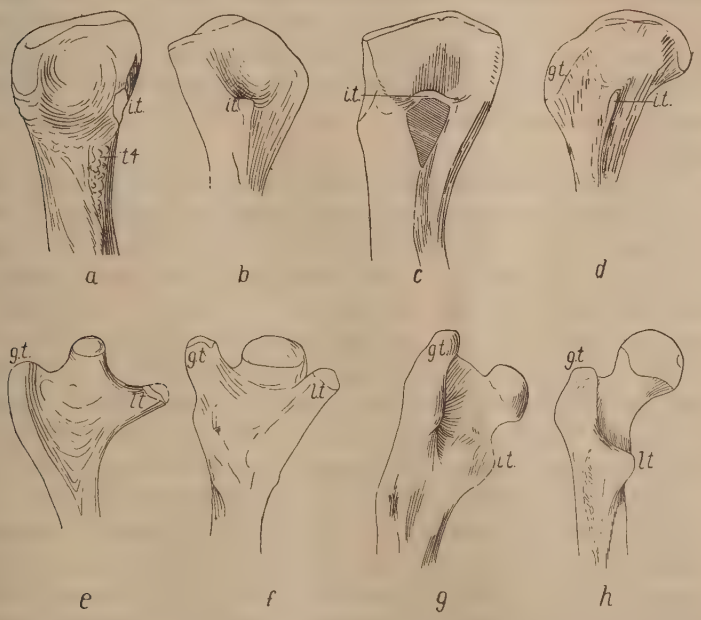

Fig. 1 Ventral (posterior) views of reptilian and mammalian femora. a, Dimetrodon (primitive reptile); b, Deuterosaurus; c, 'Dinosaurus' (primitive therapsids); d, Cynognathus (advanced therapsid); e, Jurassic mammal; f, Ornithorhynchus; g, Pachyaena (primitive placental); h, Homo (b, c, e, after Seeley; b and e reversed, g after Gregory). *i.t.*, internal trochanter; *l.t.*, lesser trochanter; *g.t.*, greater trochanter; *4t*, fourth trochanter.

lesser trochanter is rather ventral in position and direction, as is the reptilian trochanter. Both are just anterior to the 'intertrochanteric fossa.' It is reasonable to conclude, as von Huene and Gregory have done, that the two are homologous, especially since the typical mammal-like reptiles (fig. 1, d), which are intermediate in so many respects, have a single

ventral trochanter in essentially the same position. This conclusion was tentatively accepted by the writer in 1922. But, as I pointed out at that time, the reptilian and mammalian structures are radically dissimilar in their muscular relations. The reptilian internal trochanter is essentially a structure serving as a point of insertion for a portion of the homologue of the obturator externus; the mammalian lesser trochanter functions as an insertion for the iliopsoas. Further, the key to the situation is to be found in the mammal-like reptiles, and the material available to me at that time was rather scanty.

The typical mammal-like reptile (therapsid) (fig. 1, d) has a ridge on the ventral surface, somewhat anterior to the midventral line. As the only 'trochanter' present other than the greater, it might be supposed that the homologues of both the reptilian and mammalian trochanters in question may be sought in the proximal portion of this ridge, the distal portion being the linea aspera equivalent. The inquiry may be resolved into two questions: 1) Is the therapsid trochanter the homologue of the reptilian internal trochanter? 2) Is it the equivalent of the mammalian lesser trochanter?

1. The therapsid trochanter is the homologue of the reptilian internal trochanter. Prof. D. M. S. Watson, of London, has called my attention to the probable significance of the remains of very primitive therapsids from the Permian of Russia, which he has recently discussed in connection with cranial characters. The taxonomy of these forms is somewhat obscure at present, but they are apparently intermediate in many respects between American permocarboniferous types distantly related to mammals ('Pelycosaurs,' such as Dimetrodon) and the higher, more typical therapsids of South Africa. In figure 1, b and c, are illustrated femora of Russian forms. These clearly indicate the homology of the primitive trochanter (1a) with that of the higher therapsids (d). The 'intertrochanteric fossa' has been reduced, the coccygeofemoralis insertion (trochanter 4) has lost its definite character, and the inner trochanter has moved somewhat posteriorly in reaching the position it assumes in the higher therapsids.

2. The mammalian lesser trochanter is not the homologue of the therapsid trochanter. The therapsid trochanter, like its predecessor, the reptilian inner trochanter, served as the point of insertion for a portion of the obturator externus (pubo-ischiofemoralis externus). The mammalian trochanter serves as the point of insertion for iliopsoas (pubo-ischiofemoralis internus). The determination of the point of insertion of this latter muscle in the therapsid femur is hence of great importance. In most reptiles the insertion is usually in two regions, one portion of the muscle passing posteriorly over the dorsal surface of the femur to insert near the outer border and the other inserting anterodorsally fairly close to the head and tending somewhat ventrally in the distal portion of its area of insertion (Romer, '22, pl. XLVI). The former insertion, as I have shown in a previous paper, is lost in the line leading to the mammals, and need not be considered further.

If the iliopsoas were to replace the obturator externus on the trochanter existing in the therapsids, it might be expected that its point of insertion would be on the trochanter, or at least tend ventrally toward it from its originally dorsal position. Until recently I had seen no therapsid humerus which showed an indication of an iliopsoas insertion. Last summer, however, while working in the British Museum, two specimens came under my observation which shed considerable light on this question. On the dorsal surface of a cynognathid (? Diademodon) femur in the D. M. S. Watson collection a well-marked line appears proximally, running parallel to the long axis of the bone near the anterior margin, and being more conspicuous in its distal portion (fig. 2, a). This marking lies in the line of demarcation between the vasti (femorotibialis) and the iliopsoas homologue in many reptiles, and it seems clear that here as well it marked the line of demarcation between the two muscles. The main tendon of the iliopsoas probably inserted into the thickened, more distal portion. A marking somewhat similar in appearance appears on a femur of a dicynodont (a member of a collateral line of

mammal-like reptiles) in the British Museum collection (fig. 2, b). It is improbable that these markings are boundaries for the vasti, without any structure lying beyond them, or that they are indications of joint ligaments. The limits of a fleshy muscular origin are in themselves seldom defined by markings on the bone, and the lines extend too far distally to have been connected with the joint.

The mammalian lesser trochanter appears to have been primitively an outgrowth from the point of insertion of iliopsoas as defined above. This seems at first a contradiction, for the lesser trochanter of most modern mammals is ventral

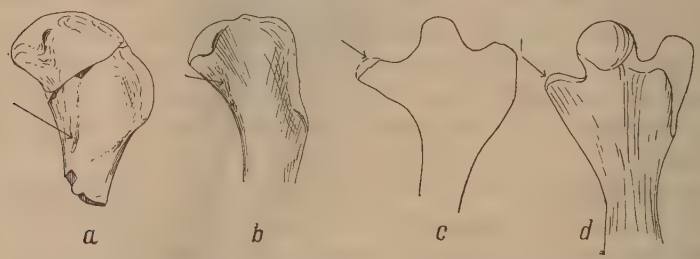

Fig. 2 Dorsal (anterior) views of therapsid and mammalian femora. a, Diademodon; b, a dicynodont (therapsids); c, Jurassic mammal (outline only); d, Ornithorhynchus. Arrow points to probable insertion of iliopsoas. (c, after Seeley.)

in position (figs. 1, h; 3, f). But in the most primitive placentals and marsupials the lesser trochanter is often directed more anteriorly than ventrally (figs. 1, g; 3, e); in the monotremes the lesser trochanter is directed anteriorly (figs. 1, f; 3, d); and this is also the case in the oldest mammalian femur known, that described by Seeley in 1879 from the jurassic Stonesfield slate (figs. 1, e; 2, c). The iliopsoas of therapsids had its main area of insertion close to the point of outgrowth of the lesser trochanter in primitive mammals. Hence the lesser trochanter appears to have been evolved, just as it has been retained, as a point of insertion for that muscle.

The reptilian internal trochanter, if the reasoning above be agreed to, is not represented in any living mammal. It is

not found in the monotremes, nor in the jurassic specimen figured. It had already undergone considerable reduction in the higher therapsids.

If the older conception of the homology of the reptilian internal and mammalian lesser trochanters be adhered to, it is difficult to explain the diverse tendencies seen in a morphological series of femora, as illustrated in figure 1 and especially in figure 3. It is necessary to believe that, from our present evidence, the evolutionary processes have reversed themselves three times in a series leading from a

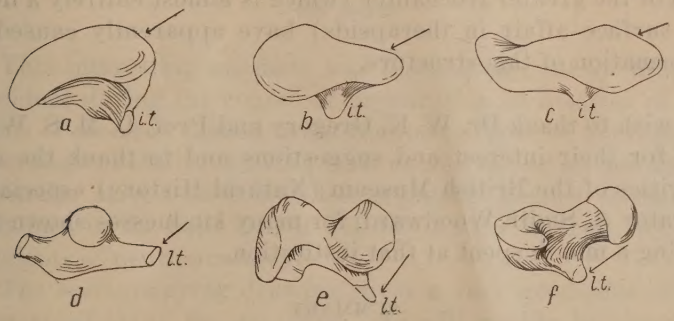

Fig. 3 Proximal views of reptilian and mammalian femora. a, Dimetrodon; b, Deuterosaurus; c, Cynognathus; d, Ornithorhynchus; e, Pachyaena; f, modernized placental. *i.t.*, internal trochanter; *l.t.*, lesser trochanter. Arow indicates insertion of iliopsoas and homologues.

primitive reptile to a typical mammal. First, the reptilian trochanter has been reduced and moved somewhat externally on the ventral surface to attain the therapsid position (figs. 1, a to d; 3, a to c). Secondly, it has enlarged and moved to the anterior border in primitive mammals (figs. 1, d, e, f; 3, c, d). Thirdly, it has again moved ventrally (figs. 1, e to h; 3, d, e, f).

If, on the other hand, the conclusion stated here be accepted, the processes involved can be easily explained. The reptilian trochanter has moved somewhat posteriorly on the ventral surface, has been reduced in therapsids, and lost altogether in mammals (figs. 1, a to e; 3, a to d). The mammalian tro-

chanter first appears at the anterior border close to the iliopsoas insertion of reptiles, and gradually moves ventrally in many mammals (figs. 1, e to h; 2, c, d; 3, d to f).

It is extremely probable that the intertrochanteric fossa is not homologous in the two classes. The old reptilian fossa is practically absent in higher therapsids, in the jurassic femur and in monotremes, not well developed in many rather primitive mammalian humeri, and only reappears in more highly developed, 'modernized' mammals. The ventral swinging of the lesser trochanter, and the development of a ventral expansion of the greater trochanter (which is almost entirely a dorsal surface affair in therapsids) have apparently caused a reformation of this structure.

I wish to thank Dr. W. K. Gregory and Prof. D. M. S. Watson for their interest and suggestions and to thank the authorities of the British Museum (Natural History) especially Curator A. Smith Woodward, for many kindnesses shown me during a month spent at that institution.

SUMMARY

The mammalian lesser trochanter is a new development in mammals, and has no reptilian predecessor.

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A PERFORATED SEPTUM PELLUCIDUM

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ONE FIGURE

This interesting anomaly was discovered entirely through accident during the course of preparation of a series of sections of an adult human brain for the purpose of microscopic neurological study. Having been unable to find an account of a similar case, I have been led to publish a short account of this one, in the hope that it will be of some interest to other students of neuro-anatomy.

The accompanying drawing gives a very good idea of the abnormalities of the specimen. As will readily be observed, the septum itself is more than twice its normal size. The fornix is accordingly displaced backward and downward, and is very imperfectly developed. The corpus callosum is larger than normal, although in one place it has become very thin. Its various parts have become noticeably distorted, and indeed it bears very little resemblance to a normal one. The cavity of the septum is obliterated and the large perforations allow free communication between the lateral ventricles.

A further abnormality, not shown in the drawing, is a slight enlargement of the lateral ventricles. However, a similar enlargement is sometimes met with in dissecting brains that are otherwise normal; hence there seems to be no connection between this enlargement and the other abnormalities.

The subject in whose brain this abnormality was found was a male of somewhat more than middle age. His last years were spent in the State Hospital for the Incurably Insane.

About six years before his death he had been struck in the head with the point of a pick, which perforated the skull in the parietal region. Afterward, on removal of the brain, it was found that the point had entered the anterior frontal convolution near the sagittal fissure. The series of sections, mentioned in the beginning, shows a limited degeneration of the great pyramidal tract of that side. However, it is greatly regretted that no record of the symptoms is available; for the actual effects of this injury, and of the abnormalities in the septum, fornix, and corpus callosum—if there were any—are entirely unknown.

Fig. 1 Medial view of cerebrum, showing perforated septum; fornix and corpus callosum abnormally developed.